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不同型間超甜玉米果穗產量與甜度之組合力探討 [1]

劉紹國 [2] 謝光照 [3,4]

摘　要

劉紹國、謝光照。2012。不同型間超甜玉米果穗產量與甜度之組合力探討。台灣農業研究 61:81–89。

為探討熱帶型與溫帶型玉米型間雜種組合果穗產量及甜度之表現，以熱帶型及溫帶型品種所分離之 9 個自交系及其 36 個半互交組合為材料，調查含苞葉果穗重與甜度性狀之平均值、組合力及雜種優勢值。不同類型間雜交組合果穗產量及籽粒糖度之比較，顯示含苞葉果穗鮮重為熱帶型 × 熱帶型 ≥ 熱帶型 × 溫帶型 > 溫帶型 × 溫帶型；其中高於 400 g 者的熱帶型 × 溫帶型組合有 PH 5-5 × GH 8、PH 10-23 × GH 8、PH 10-23 × Venus 8、PH 10-23 × Honey 236-1、PH 10-23 × HJ 2-7、PH 10-23 × SN 3-8 等 6 個組合。甜度在型間無明顯差異，高於 15°Brix 的組合，在熱帶型 × 溫帶型中有 BJ 12 × Venus 8、PH 5-5 × SN 3-8、PH 10-23 × SN 3-8、SH-1 × 金蜜 8 等 4 個組合。整體而言，熱帶型自交系一般帶有較多增加鮮果穗產量之有利基因；而溫帶型自交系則帶有較多增加甜度之有利基因。因此熱帶型 × 溫帶型之組合具有互補作用，可作為超甜玉米育種上理想之雜種優勢模式群。

關鍵詞：超甜玉米、熱帶型、溫帶型、雜種優勢、組合力。

前　言

甜玉米為美國、日本、韓國和我國等國民眾所廣泛食用，目前美國是世界上最大甜玉米生產和出口國，甜玉米總產值排在鮮售蔬菜產品的第 4 位和加工產品的第 2 位。全世界甜玉米種植面積約 113.3 萬公頃，美國種植面積約 33 萬公頃，其中約 70% 供作鮮食及 30% 的加工產品進入國際市場。超甜玉米以鮮食為主，除重視鮮穗產量外，果穗形態、甜度、果皮厚度、口感等也都是育種者所關心的品質性狀。

在台灣飼料玉米遺傳與育種研究，顯示溫帶型馬齒種與熱帶型硬粒種種原間具有優越的雜種優勢及高度的特殊組合力 (Lee *et al*. 1986; Shieh & Thseng 1993)，同時可應用此溫帶型馬齒種與熱帶型硬粒種間之雜種優勢模式於生產高產的單雜交玉米品種 (Wan *et al*. 1986; Lee *et al*. 1989)。

玉米產量和品質決定於基因型和環境因

1. 行政院農業委員會農業試驗所研究報告第 2662 號。接受日期：101 年 3 月 23 日。
2. 行政院農業委員會農糧署蔬菜花卉科技正。台灣　南投縣。
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素。而甜玉米的品質性狀主要分為兩個部分，一是果穗的外觀品質，二是食用和加工品質。普遍認為甜玉米食用和加工品質，其主要決定因子是籽粒含糖量與組成分、水溶性多糖的含量 (sweetness)、果皮柔嫩度 (tenderness)、香味和含水量等，而果皮柔嫩度與果皮厚薄及含量有關 (Ito & Brewbaker 1981)。甜玉米的糖分含量是決定品質的最重要因素，Wu *et al*. (1999) 研究指出甜玉米籽粒含糖量是數量性狀遺傳，符合加性-顯性遺傳模型，其中加性效應對含糖量性狀的表現起主導作用，且此性狀的遺傳力較高，在自交的早期世代選拔是有效的；而 Wang *et al*. (2005) 研究亦顯示超甜玉米含糖量之遺傳也符合加性-顯性模型，而加性效應在含糖量遺傳效應中居於主導作用，雜種優勢的表現為中親優勢且具有正向增加優勢效應，已知至少受 3 對基因控制，其狹義遺傳率約 53%。而其他學者針對甜玉米的品質性狀遺傳研究顯示籽粒含糖量受加性和非加性效應共同作用，其遺傳力較低，同時也受到細胞質基因的控制 (Luo & Wu 2005)。Wang *et al*. (2004) 以 7 個超甜型玉米自交系為親本，研究超甜型玉米蔗糖含量的組合力效應，指出蔗糖含量的遺傳受加性基因和非加性基因共同控制；各品質性狀的一般組合力效應、特殊組合力效應及其變異幅度均存在顯著差異。

綜合言之本研究主要是探討溫帶型與熱帶型玉米型間鮮果穗產量與甜度是否有雜種優勢模式存在，及對品質之影響是否一致，期望能在栽培或育種方面提供有用的訊息，供玉米栽培研究及品種改良上之參考。

材料與方法

以分別分離自不同商業品種之 9 個 S5 之自交系及其半互交之 36 個 F_1 組合為材料，進行鮮果穗產量 (含苞葉果穗鮮重) 及籽粒甜度性狀之遺傳分析。其中有 4 個為熱帶型自交系，分別為 PH 5-5、BJ 12、PH 10-23 及 SH-1；5 個為溫帶型自交系，GH 8、Venus 8、Honey 236-1、HJ 2-7 及 SN 3-8 (表 1)。田間試驗在 2007 年 11 月於南投縣集集鎮農家田間進行。田間設計為逢機完全區集設計 (Randomized Complete Block Design, RCBD)，三重覆，行長 4.5 m，3 行區，行株距為 80 cm × 30 cm。三要素肥料量為 N：P_2O_5：K_2O = 180：90：60 kg/ha，基肥以台肥 39 號複合肥料 (N：P_2O_5：K_2O = 12：18：12) 每公頃施 500 kg，即基肥每公頃施氮素 60 kg，磷肥 90 kg 及鉀肥 60 kg。其餘之氮素 120 kg 以硫酸銨 (N = 21%) 於玉米長至齊膝期 (約 50–60 cm) 當追肥施

表 1. 試驗材料一覽表

Table 1. Nine inbred lines of super sweet corn used as parents of diallel crosses in the study

Inbred line	Type	Company or institute
PH 5-5	Tropical	TAIPEI NONG CI CO. Taiwan
BJ 12	Tropical	KNOWN-YOU SEED CO.,LTD. Taiwan
PH 10-23	Tropical	TAIPEI NONG CI CO. Taiwan
SH-1	Tropical	Taiwan Agricultural Research Institute (TARI)
GH 8	Temperate	BO YOU CO., LTD. Taiwan
Venus 8	Temperate	BO YOU CO., LTD. Taiwan
Honey 236-1	Temperate	SINON CORPORATION. Taiwan
HJ 2-7	Temperate	KNOWN-YOU SEED CO., LTD. Taiwan
SN 3-8	Temperate	SINON CORPORATION Taiwan

用。其餘之田管理按實際需要進行之。

生育期間調查含苞葉果穗重及甜度。調查方法如下：(1) 含苞葉果穗鮮重 (ear fresh weight with husk)：逢機選取乳熟期之五個果穗，稱其含苞葉果穗鮮重，以 g/ear 表示。(2) 甜度 (sugar content)：取果穗中間段之籽粒擠出汁液，以糖度計測其糖度，以°Brix 表示。

所得數據以 SAS 中 General Linear Model Procedure 進行變方分析，並進行平均值顯著性比較，及以 Griffing (1956) 模式 I 方法二進行組合力分析。

結　果

以 9 個商業品種分離至 S5 之自交系及半互交之 36 個 F_1 組合為材料進行含苞葉果穗鮮重及甜度之調查。分析各性狀之變異、平均值、組合力變方分析之均方值、親本之 GCA 效應值、雜交種的 SCA 效應值、平均雜種優勢值等，相關結果表現敘述如下：

F_1 含苞葉果穗鮮重平均值之變異幅度在 166.7–472 g/ear，平均為 333.06 g/ear (表 2)，最低為 Venus 8 × Honey 236-1，最高為 PH 5-5 × PH 10-23。低於 300 g 的組合有 SH-1 × Venus 8、GH 8 × Venus 8、GH 8 × Honey 236-1、GH 8 × HJ 2-7、GH 8 × SN 3-8、Venus 8 × Honey 236-1、Venus 8 × HJ 2-7、Venus 8 × SN 3-8、Honey 236-1 × HJ 2-7 及 Honey 236-1 × SN 3-8 等 10 個組合。高於 400 g 者有 PH 5-5 × PH 10-23、PH 5-5 × SH-1、PH 5-5 × GH 8、PH 10-23 × SH-1、PH 10-23 × GH 8、PH 10-23 × Venus 8、PH 10-23 × Honey 236-1、PH 10-23 × HJ 2-7、PH 10-23 × SN 3-8 等 9 個組合。

含苞葉果穗鮮重經組合力變方分析顯示 (表 3)，組合間之特殊組合力及自交系間一般組合力皆呈極顯著差異。各品種一般組合力效應值變異幅度從 -45.17 至 90.06 間 (表 4)，由低至高依序為 Honey 236-1、Venus 8、SN 3-8、GH 8、HJ 2-7、BJ 12、SH-1、PH 5-5 及 PH 10-23，熱帶型自交系的一般組合力效應多為正值，而溫帶型自交系的一般組合力效應多為負值。含苞葉果穗鮮重不同組合間特殊組合力效應值變異幅度從 -66.53 至 78.45 間 (表 4)，效應值為負者有 PH 5-5 × BJ 12、BJ 12 × PH 10-23、BJ 12 × SH-1、PH 10-23 × SH-1、GH 8 × HJ 2-7 及 GH 8 × SN 3-8 等 8 個組合。

F_1 含苞葉果穗鮮重雜交組合間平均雜種優勢值 (%) 變異幅度從 57.7 至 317.0% 間 (表 5)，小於 100 者有 SH-1 × PH5-5、SH-1 ×

表 2. 超甜玉米品種間雜交組合含苞葉果穗鮮重之平均值 (g/ear)

Table 2. Fresh ear weight with husk (g/ear) of 36 hybrids from crosses of nine inbred line of super sweet corn

Inbreds	PH 5-5	BJ 12	PH 10-23	SH-1	GH 8	Venus 8	Honey 236-1	HJ 2-7	SN 3-8
PH 5-5	193.6	324.3	472.0	412.0	401.6	367.6	352.3	366.2	374.3
BJ 12		106.9	380.0	303.6	371.0	327.0	337.0	348.0	310.0
PH 10-23			201.6	402.6	465.0	447.0	417.5	428.6	436.0
SH-1				220.0	334.0	296.3	322.3	362.0	330.0
GH 8					71.0	261.3	264.1	260.4	242.0
Venus 8						116.8	166.7	263.3	260.0
Honey 236-1							94.5	226.3	252.6
HJ 2-7								159.3	276.3
SN 3-8									71.0

$LSD_{0.05}$ = 20.1

BJ12、SH-1 × PH10-23、Venus 8 × SH-1、Honey 236-1 × Venus 8、HJ 2-7 × SH-1、HJ 2-7 × Venus 8、HJ 2-7 × Honey 236-1 等 8 個組合；大於 200 以上者為 GH 8 × PH 5-5、GH 8 × BJ 12、GH 8 × PH 10-23、Honey 236-1 × BJ 12、SN 3-8 × BJ 12、SN 3-8 × PH 10-23、SN 3-8 × GH 8 及 SN 3-8 × Honey 236-1 等 9 個組合。

含苞葉果穗鮮重型間平均值之比較，呈現熱帶型 × 熱帶型 ≥ 熱帶型 × 溫帶型 > 溫帶型 × 溫帶型；熱帶型一般組合力效應平均值為正值 (37.85 g/ear)，而溫帶型一般組合力效應平均值為負值 (-29.90 g/ear)；不同型間特殊組合力效應值之比較，呈現熱帶型 × 熱帶型及溫帶型 × 溫帶型為負值，而熱帶型 × 溫帶型為正值；型間之平均雜種優勢值，熱帶型 × 溫帶型表現最大，次之溫帶型 × 溫帶型，最小為熱帶型 × 熱

表 3. 超甜玉米品種間雜交組合含苞葉果穗鮮重組合力變方分析之均方值

Table 3. Mean square of fresh ear weight with husk in 9 × 9 diallel set of super sweet corn

Source	Df	MS
GCA	8	35238**
SCA	35	12184**
Error	134	828
GCA/SCA[z]		2.89

[z] GCA: General combining ability; SCA: Specific combining ability.
** Significant at 0.01 probability level.

表 4. 超甜玉米各自交系與雜交 F_1 含組合苞葉果穗鮮重之 GCA (對角線) 和 SCA 效應值 (上三角) (g/ear)

Table 4. Eastimated values of general and specific combining ability effects of fresh ear weight with husk of 36 hybrids in 9 × 9 diallel set

Inbreds	PH 5-5	BJ 12	PH 10-23	SH-1	GH 8	Venus 8	Honey 236-1	HJ 2-7	SN 3-8
PH 5-5	47.10	-34.80	19.23	33.37	57.67	42.08	34.81	20.19	43.55
BJ 12		-3.56	-21.75	-24.28	77.68	52.08	70.15	52.29	29.89
PH 10-23			90.06	-18.91	78.05	78.45	57.08	39.63	62.26
SH-1				15.93	21.85	1.92	35.99	47.10	30.40
GH 8					-18.70	1.56	12.46	-19.82	-22.29
Venus 8						-37.11	-66.53	1.48	13.44
Honey 236-1							-45.17	-27.45	14.17
HJ 2-7								-16.62	9.28
SN 3-8									-31.92

S.E. (G_i-G_j) = 9.59
S.E. $(S_{ij}-S_{ik})$ = 27.13
S.E. $(S_{ij}-S_kl)$ = 25.38

表 5. 超甜玉米品種間雜交組合含苞葉果穗鮮重之平均雜種優勢值 (%)

Table 5. Heterosis of 36 hybrids for fresh ear weight with husk of super sweet corn

Inbreds	PH 5-5	BJ 12	PH 10-23	SH-1	GH 8	Venus 8	Honey 236-1	HJ 2-7
BJ 12	115.8							
PH 10-23	138.7	146.5						
SH-1	99.1	85.7	90.9					
GH 8	203.5	317.0	241.0	130.0				
Venus 8	136.8	192.3	180.6	75.9	178.2			
Honey 236-1	144.5	234.6	181.9	104.9	219.1	57.7		
HJ 2-7	107.5	161.4	137.4	90.6	126.1	90.7	78.3	
SN 3-8	182.8	248.5	219.8	126.8	241.7	176.8	205.7	139.9

帶型 (表6)。

籽粒甜度平均值變異幅度在 12.0–16.3°Brix 間，平均值為 14.1°Brix (表 7)，最低者為 PH 10-23 × SH-1，最高者為 GH 8 × SN 3-8。低於 13 者僅 PH 10-23 × SH-1一個組合。高於 15 者有 BJ 12 × Venus 8、BJ 12 × SN 3-8、PH 10-23 × SN 3-8、SH-1 × GH 8、GH 8 × Venus 8、GH 8 × SN 3-8 及 Honey 236-1 × SN 3-8 等 7 個組合。經組合力變方分析顯示 (表 8)，籽粒甜度在自交系間之一般組合力呈極顯著差異。各自交系的一般組合力效應值變異幅度從 -0.03 至 1.00 間 (表 9)，僅 SN 3-8 為正值，其餘皆為負值，由低至高依序為 PH 5-5、BJ 12、PH 10-23、Venus 8、Honey 236-1、SH-1、GH 8 及 HJ 2-7。不同雜交種間特殊組合力效應值變異幅度從 -1.396 至 1.600 間 (表 9)，效應值小於 -1 者為 PH 10-23 × SH 1，大於 1 者為 BJ 12 × Venus 8、SH-1 × GH 8、SH-1 × Honey 236-1 及 Honey 236-1 × SN 3-8 等 4 個組合。雜種間平均雜種優勢值 (%) 變異幅度從 -10.23 至 19.43 間 (表 10)，小於 -5 者有 GH 8 × PH 5-5、HJ 2-7 × GH 8 及 SN 3-8 × PH 5，大於 10 以上者為 Venus 8 × BJ 12、Venus 8 × PH 10-23、Venus 8 × SH-1、Honey 236 × SH-1 及 SN 3-8 號 × Honey 236-1。

甜度之平均值表現在型間呈現無明顯差異，熱帶型 × 溫帶型及溫帶型 × 溫帶型具有較大值，而熱帶型 × 熱帶型具有較小值。熱帶型一般組合力效應平均值為負值，溫帶型一般組合力效應平均值為正值；不同型間組合特殊組合力效應值在熱帶型 × 溫帶型為正值，而熱帶型 × 熱帶型及溫帶型 × 溫帶型為負值；型間之平均雜種優勢值，熱帶型 × 溫帶型為最大值，其次為溫帶型 × 溫帶型，最小為熱帶型 × 熱帶型 (表 11)。

表 6. 甜玉米不同型間含苞葉果穗鮮重之平均值、GCA、SCA 及平均雜種優勢值

Table 6. The means, general combining ability (GCA), specific combining ability (SCA), and heterosis of fresh ear weight with husk in different type super sweet corn

Type	Mean (g/ear)	GCA (g/ear)	SCA (g/ear)	Heterosis (%)
Tropic		37.38		
Temperate		-29.90		
Tropic × Tropic	382.2		-7.86	112.78
Tropic × Temperate	369.6		46.67	170.89
Temperate × Temperate	247.3		-8.37	151.42

表 7. 超甜玉米品種間雜交組合甜度之平均值 (°Brix)

Table 7. Means of sugar content (°Brix) of 36 hybrids from crosses of nine inbred line

Inbreds	PH 5-5	BJ 12	PH 10-23	SH-1	GH 8	Venus 8	Honey 236-1	HJ 2-7	SN 3-8
PH 5-5	15.6	14.3	13.9	13.2	13.9	14.4	14.1	13.8	13.6
BJ 12		13.4	14.8	13.6	13.9	16.1	13.7	13.4	15.3
PH 10-23			13.1	12.0	14.6	14.8	13.2	13.7	15.8
SH-1				11.7	15.8	14.4	14.6	13.1	14.3
GH 8					15.9	15.8	13.9	13.3	16.3
Venus 8						12.9	13.3	14.6	14.9
Honey 236-1							12.2	13.0	15.8
HJ 2-7								13.3	13.9
SN 3-8									16.1

$LSD_{0.05}$ = 1.00

討　　論

玉米籽粒產量和品質決定於基因型和環境因素。本試驗調查超甜玉米在含苞葉鮮果穗產量之表現，結果顯示溫帶型與熱帶型不同型間雜交組合優於同型內之組合，呈現溫帶型 × 熱帶型 ≥ 熱帶型 × 熱帶型 > 溫帶型 × 溫帶型之結果。與 Liu *et al*. (2009) 以品種間之雜交組合為材料探討雜種優勢類群之結果，顯示去苞葉果穗重呈現熱帶型 × 熱帶型相近於溫帶型 × 熱帶型大於溫帶型 × 溫帶型之趨勢，表示熱帶型 × 熱帶型及溫帶型 × 熱帶型之雜交組合具較高之產量雜種優勢之結果相類似。其中表現高產量者 (高於 400 g/ear) 如 PH 5-5 × PH 10-23、PH 5-5 × SH-1、PH 5-5 × GH 8、PH 10-23 × SH-1、PH 10-23 × GH 8、PH 10-23 × Venus 8 、PH 10-23 × Honey 236-1、PH 10-23 × HJ 2-7 及 PH 10-23 ×

表 8. 超甜玉米品種間雜交組合甜度組合力變方分析之均方值

Table 8. Mean square of analysis of combining ability for sugar content in different types of super sweet corn

Source	Df	MS
GCA[z]	8	5.638**
SCA	35	1.289**
Error	134	1.146
GCA/SCA		4.4

[z] GCA: General combining ability; SCA: Specific combining ability.
** Significant at 0.01 probability level.

表 9. 超甜玉米各自交系之 GCA 效應值 (對角線) 與雜交 F_1 含組合甜度之 SCA 效應值 (上三角) (°Brix)

Table 9. Eastimated value of general and specific combining ability effects of sugar content of 36 hybrids in 9 × 9 diallel set

Inbreds	PH 5-5	BJ 12	PH 10-23	SH-1	GH 8	Venus 8	Honey 236-1	HJ 2-7	SN 3-8
PH 5-5	-0.03	0.03	-0.02	-0.44	-0.89	-0.18	0.35	0.29	-0.53
BJ 12		-0.04	0.75	-0.11	-0.92	1.49	-0.09	-0.21	0.01
PH 10-23			-0.24	-1.40	-0.04	0.43	-0.31	0.37	0.82
SH-1				-0.59	1.60	0.44	1.47	0.15	-0.37
GH 8					-0.59	0.60	-0.44	-0.86	0.49
Venus 8						-0.35	-0.80	0.64	-0.64
Honey 236-1							-0.47	-0.13	1.05
HJ 2-7								-0.65	-0.67
SN 3-8									1.00

S.E. $(G_i\text{-}G_j) = 0.356$
S.E. $(S_{ij}\text{-}S_{ik}) = 1.009$
S.E. $(S_{ij}\text{-}S_{jk}) = 0.944$

表 10. 超甜玉米品種間雜交組合甜度之平均雜種優勢值 (%)

Table 10. The heterosis (%) of sugat content for F1 hybrids in different type of super sweet corn

Inbreds	PH 5-5	BJ 12	PH 10-23	SH-1	GH 8	Venus 8	Honey 236-1	HJ 2-7
BJ 12	-1.21							
PH 10-23	-2.54	9.92						
SH-1	-2.98	7.09	-2.88					
GH 8	-10.23	-4.10	0.20	12.96				
Venus 8	0.74	19.43	11.50	14.75	8.32			
Honey 236-1	1.26	6.31	3.75	19.33	-0.73	5.14		
HJ 2-7	-3.74	0.32	3.06	4.24	-7.82	9.45	1.47	
SN 3-8	-6.48	3.28	7.21	2.41	1.85	2.62	10.42	-4.78

表 11. 甜玉米籽粒甜度在不同型間之平均值、GCA、SCA 及平均雜種優勢值

Table 11. The means, general combining ability (GCA), specific combining ability (SCA), and heterosis of sugar content in different types of super sweet corn

Type	Mean (g/ear)	GCA (g/ear)	SCA (g/ear)	Heterosis (%)
Tropic		-0.20		
Temperate		0.16		
Tropic × Tropic	13.6		0.20	1.23
Tropic × Temperate	14.3		0.19	5.54
Temperate × Temperate	14.3		0.08	2.59

SN 3-8，皆為溫帶型 × 熱帶型或熱帶型 × 熱帶型之組合，惟熱帶型中之華珍 12 自交系與熱帶型或溫帶型之組合表現相對較差，其 GCA 效應值為 -3.56，與其他溫帶型品種同屬負值，PH 10-23 之 GCA 效應值為 90.06 最高，與各品種之組合表現最佳；溫帶型品種 GCA 效應值皆為負值。SCA 效應值呈現溫帶型 × 熱帶型 > 熱帶型 × 熱帶型 ≥ 溫帶型 × 溫帶型之趨勢，其中 PH 5-5 × GH 8、BJ 12 × GH 8、BJ 12 × Venus 8、BJ 12 × Honey 236-1、BJ 12 × HJ 2-7、PH 10-23 × GH 8、PH 10-23 × Venus 8、PH 10-23 × Honey 236-1 及 PH 10-23 × SN 3-8 組合有較高的 SCA 效應值。自交系 PH 10-23 及 PH 5-5 之 GCA 效應值表現皆佳，BJ 12 及 PH 10-23 則有較佳的 SCA 效應值表現。而雜種優勢值表現亦以熱帶型 × 溫帶型為佳，但溫帶型 × 溫帶型之中 GH 8 × Honey 236-1、GH 8 × SN 3-8 及 SN 3-8 × Honey 236-1 之組合也表現高的雜種優勢值，可以作為甜玉米產量性狀的重要種質。

在甜度平均值之表現，顯示超甜玉米各種型間無明顯差異，熱帶型 × 熱帶型組合中無高於 15°Brix 的組合；而熱帶型 × 溫帶型高於 15°Brix 的組合有 BJ 12 × Venus 8、PH 5-5 × SN 3-8、PH 10-23 × SN 3-8 及 SH-1 × GH 8 等；溫帶型 × 溫帶型高於 15 的組合有 GH 8 × Venus 8、GH 8 × SN 3-8 及 Honey 236-1 × SN 3-8。平均而言，甜度仍以不同型間之組合表現佳，而雜交組合 GCA 均方值呈極顯著差異，SCA 則無顯著差異存在，親本之 GCA 效應值僅 SN 3-8 為正值 (0.99)，雜交組合 SCA 效應值無顯著差異存在。本試驗與 Wu *et al*. (1999) 及 Wang *et al*. (2005) 研究指出含糖量性狀是數量性狀遺傳，加性效應對含糖量性狀的表現起主導作用之結論相符合。又雜種優勢值在型間組合存在差異，以溫帶型 × 熱帶型 > 溫帶型 × 溫帶型 > 熱帶型 × 熱帶型之趨勢表現，其中以溫帶型的 Venus 8 與熱帶型間之組合有較高之雜種優勢值。

綜合本試驗由自交系間之組合力分析之結果，顯示超甜玉米種原存在溫帶型與熱帶型間之產量雜種優勢模式，且熱帶及亞熱帶玉米具有豐富的遺傳多樣性與溫帶型玉米種質交流少，遺傳變異較大，溫帶與熱帶異型間之雜交組合，不論在一般組合力或特殊組合力上，皆大於同型間之組合，可產生較強的雜種優勢，大幅提高產量 (He *et al*. 2005)，與 Li *et al*. (2001)、Zhang *et al*. (2006)、Sun *et al*. (2007) 在中國大陸試驗結果呈現不同型間具雜種優勢，主要的雜種優勢模式皆屬溫帶型與熱帶型的組合報告相類似。即熱帶型與溫帶型間產量，熱帶型品種為正效應居多，溫帶型品種為負效應居多；而籽粒甜度性狀，熱帶型品種為負效應居多，溫帶型品種為正效應居多。故溫帶型 × 熱帶型之雜種優勢模式群可以充份利用於超甜玉米育種上材料的選擇與組配上，以建立適合於亞熱帶台灣之基礎種原材料，並可供育種之參考與利用。

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Combining Ability of Ear Yield and Sugar Content in Different Types of Super-Sweet Corn[1]

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Abstract

Liu, S. K. and G. J. Shieh. 2012. Combining ability of ear yield and sugar content in different types of super-sweet corn. J. Taiwan Agric. Res. 61:81–89.

A field experiment was conducted during 2007 to determine combining ability of fresh ear weight and sugar content in F_1 hybrids of super-sweet corn. Cross were made using four inbred lines of tropical type and five inbred lines of temperate type to produce 36 F_1 hybrids for testing in the field to compare fresh ear yield (with husk) and sugar content by analysis of a half diallel set. Results showed that the fresh ear weight with husk was higher in hybrids of tropical type × tropical type and tropical type × temperate type than in hybrids of temperate type × temperate type. There was no significant difference ($P > 0.05$) in sugar content among hybrids of tropical type × tropical type and tropical type × temperate type. In general, the tropical inbred lines had more positive genes for the fresh ear weight, and the temperate inbred lines generally had more positive genes for sugar content. Therefore, production of hybrids by tropical type inbreds and temperate type of inbreds is a promissing strategy for breeding super sweet corn.

Key words: Super sweet corn, Tropical type, Temperate type, Heterotic pattern, Combining ability.

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台灣農業研究 (J. Taiwan Agric. Res.) 61(2):90–99 (2012)

稻草在秀珍菇栽培之應用[1]

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摘　要

李瑋崧、呂昀陞、陳美杏。2012。稻草在秀珍菇栽培之應用。台灣農業研究 61:90–99。

秀珍菇是台灣五大栽培菇類之一，平均每年用於秀珍菇太空包生產的木屑量超過 3 萬公噸，為求菇類產業與環境生態之永續發展，本研究開發稻草取代部分木屑栽培秀珍菇之模式。比較秀珍菇 6 個菌株在馬鈴薯葡萄糖洋菜培養基之菌絲生長情形，得知 SJ-10 菌株生長速度最快，而 SJ-11 菌株生長速度最慢。為了解不同菌株對於稻草之利用性，選取 SJ-10 和 SJ-11 兩菌株進行稻草與木屑不同乾重混合比例之菌絲生長與出菇試驗。結果經變方分析後顯示菌株與不同稻草與木屑混合比例對菌絲生長、產量、生物效率與生物轉化率之交感效應極顯著 ($P < 0.01$)，表示不同菌株會隨著稻草與木屑不同混合比例而有差異表現。在菌絲生長方面，兩菌株在稻草與木屑混合比例 1：3 之基質和全木屑處理者生長最快。在出菇試驗方面，SJ-10 菌株培養於稻草與木屑混合比例 1：1 之處理，與 SJ-11 菌株培養於稻草與木屑混合比例 1：3 之處理，無論在產量、生物效率和生物轉化率均顯著高於全木屑處理者；SJ-10 菌株以稻草與木屑混合比例 1：1 與 SJ-11 菌株以稻草與木屑混合比例 1：3 培養的平均每包產量分別為 192.3 g 和 222.5 g。評估以稻草應用於秀珍菇栽培模式之收益，結果顯示 SJ-10 菌株以稻草與木屑混合比例 1：1 處理者與 SJ-11 菌株以稻草與木屑混合比例 1：3 處理者均較全木屑處理者分別多出 15000 元/萬包和 22500 元/萬包。綜合上述研究，利用稻草取代部分木屑栽培秀珍菇，不僅可降低成本，更能提高產能增加收益。

關鍵詞：秀珍菇、稻草、產量、生物效率、生物轉化率。

前　言

秀珍菇 [*Pleurotus sajor-caju* (Fr.) Singer] 原產於印度，英文名為 Indian mushroom，因其菇體形狀又稱為鳳尾菇 (Phoenix-tail mushroom)，最早是在喜馬拉雅山腳的 *Euphorbia royleans* 樹上被發現 (Chang *et al*. 1981)，在印度普遍被栽培和食用 (Basak *et al*. 1996)，其菇柄細長，菇傘成扇形，模樣討喜又容易烹調，深受台灣消費者喜愛，因而躍身為國內五大栽培菇類之一，栽培量僅次於香菇、金針菇、杏

1. 行政院農業委員會農業試驗所研究報告第 2663 號。接受日期：101 年 3 月 23 日。
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鮑菇和木耳。國內栽培方式主要以木屑太空包栽培，平均每年生產約 3000 萬包，大約需用掉 3.6 萬公噸木屑，近年因環保意識抬頭與人造林木供應緩慢，造成木屑來源逐漸短缺導致價格上漲，現今每公斤新鮮木屑售價已上漲 5 成以上，使得國內菇農面臨了原料匱乏和生產成本增加之雙重壓力，為使菇類產業成為與環境友善的事業並永續發展，積極開發可供菇類生長之木屑替代基質已是刻不容緩。替代基質須符合容易取得和可以穩定供應兩個要件，若能善加利用大宗農業廢棄物於菇類栽培，對整體菇類產業之永續經營，及對環境資源之節約與再利用都是相當重要的。

水稻是台灣種植面積最廣的作物，據行政院農業委員會農業統計要覽 (COA 2011) 之統計，國內水稻年產稻穀量雖自 2005 年的 146.7 萬公噸減少到 2010 年的 145.1 萬公噸，若以稻草和稻穀重量約為 1：1 推估，台灣每年仍可生產 140 萬公噸以上稻草，且農民處理稻草常用就地焚燒方式，造成空氣環境污染的情況發生。稻草富含木質素和纖維素 (Boonmee 2009; Choudhary *et al*. 2009)，在國外發展利用稻草 (Murugesan *et al*. 1995; Ragunathan *et al*. 1996; Muthukrishnan *et al*. 2000; Shashirekha *et al*. 2002; Nageswaran *et al*. 2003; Bonatti *et al*. 2004; Poppe 2004) 或麥稈 (Zadražil 1980; Royse & Schisler 1987; Tsang *et al*. 1987; Moyson & Verachtert 1991; Royse *et al*. 1991; Royse 2003) 栽培蠔菇屬 (genus *Pleurotus*) 菇類 (Philippoussis *et al*. 2001; Velázquez-Cedeño *et al*. 2002; Mane *et al*. 2007) 之模式較早，秀珍菇為蠔菇屬的成員之一，可以分解和利用木質素 (Kurt & Buyukalaca 2010; Singh *et al*. 2011)、纖維素 (Basak *et al*. 1996) 或半纖維素 (Royse 1992; Awafo *et al*. 1995) 等物質，且具有非常高的腐生盤據能力 (Zadražil 1980)。Ragunathan *et al*. (1996) 以稻草 (rice straw)、玉米稈 (maize stover)、蔗渣 (sugarcane bagasse)、椰子殼髓 (coir pith) 等四種農業廢棄物栽培秀珍菇，結果指出以稻草為基質栽培秀珍菇的產量最高，每公斤乾物質可生產 0.396 kg 鮮菇。

國內生產秀珍菇的太空包製作採半機械化方式，先以機器將所有材料攪拌均勻後自動裝填至塑膠袋中，再以人工套上塑膠環及棉塞，如果直接使用自水稻田收取的稻草，或是切成一吋長度之稻草，均會阻塞機器而難以應用在現行製包系統，如果全以人工裝填，則會大幅增加生產成本，因而限制了稻草在菇類栽培上的應用。本所在 2009 年以機器切割稻草至長度僅約 1 cm，已成功地利用太空包製作機將稻草與其他材料混合裝填入塑膠袋中。因此，本研究的主要目的乃利用木屑與稻草不同乾重混合比例種植秀珍菇，評估以稻草替代木屑應用於國內秀珍菇栽培模式的可行性，期能有效降低生產成本和減少栽種菇類所需的木屑用量。

材料與方法

試驗菌株之收集與保存

自台灣各地秀珍菇栽培場共蒐集 SJ-05、SJ-06、SJ-09、SJ-10、SJ-11 和 SJ-12 六個菌株，分別培養於馬鈴薯葡萄糖洋菜培養基 (potato dextrose agar, PDA; Difco) 試管斜面上，待菌絲長滿斜面後保存於 4℃ 冰箱，定期做繼代培養。

比較秀珍菇不同菌株之菌絲生長情形

將上述秀珍菇菌株培養在 PDA 平板培養基上，置於室溫培養六天後，以消毒過的打孔器截取菌落邊緣的菌絲塊 (直徑 5 mm)，之後以消毒過之移植針將其分別移植到 PDA 平板培養基中間 (培養皿直徑 9 cm)，每一處理 6 個培養皿，然後放置於 26℃ 定溫箱中培養六天後，量測菌落直徑，比較不同菌株間菌絲生長速度。

菌種製作

將麥粒加水煮熟，瀝乾後混合 1% (w/w) 之碳酸鈣，裝入四角玻璃瓶中，經高溫高壓 (121℃, 1.2 kg/cm^2) 滅菌後備用。將前述長滿 PDA 培養基斜面之秀珍菇菌絲塊以移植針移入滅菌過之麥粒中，於 24℃ 定溫、無光照培養至菌絲長滿，再將長滿菌絲之麥粒以接種棒移入滅菌過之瓶裝木屑栽培基質 [鋸木屑與米糠 10：1 (v/v) 混合，含水量為 65% 左右]，於 24℃ 定溫、無光照培養至菌絲長滿瓶內基質為止。

太空包栽培基質之製作

以木屑 (新鮮山黃麻木屑加水，經堆積 3 個月後，期間每個月翻堆一次) 和稻草分別以乾重比 0：1、1：3、1：1、3：1 和 1：0 之比例的混合，共 5 種處理。每一處理均添加基質總乾重 20% 米糠和 25% 粉頭 (wheat bran)，並以碳酸鈣調整 pH 值為 6.5。所有材料加入基質混合槽中攪拌均勻後，調整含水量至 60–63% (各配方滅菌後之 pH 質與含水量如表 1 所示)，以太空包裝填機 (忠興鐵工廠，台灣) 裝袋 (聚丙烯塑膠袋，9 cm × 8 cm × 34 cm)，每包裝填 900 g 基質 (濕重)，打洞、加塑膠套環、塞好棉塞，放入塑膠框中，每框 12 包，並於棉塞上面覆上一層錫箔紙以防止滅菌中棉塞受潮，然後移入電腦自動化程式控制之殺菌釜 (廣太原，台灣) 內滅菌 (121℃，1 小時)。

秀珍菇 SJ-10 和 SJ-11 菌株在不同稻草和木屑混合比例之菌絲生長情形

為了比較不同秀珍菇菌株對於稻草之利用性，選取上述 6 個菌株中生長速度最快的菌株 (SJ-10) 和最慢的菌株 (SJ-11) 分別作為不同稻草與木屑混合比例之試驗菌株。以前述 5 種不同比例混合之稻草與木屑為材料，各配方分別秤取 150 g 放入直徑 14 cm 之玻璃培養皿，不同菌株以不同配方之處理組合各 4 重複 (培養皿)。將培養基質壓至相同高度以確保基質密度均一，並於正中央嵌入直徑 3 cm 之聚丙烯塑膠環 (使其有高於料面約 1 cm 之空間以便接種)，經高溫高壓滅菌後放冷，以人工定量接種秀珍菇二品系之木屑菌種於聚丙烯塑膠環內，於 24℃ 定溫、無光照培養 10 天後，量測菌落直徑。

秀珍菇在不同稻草與木屑混合比例基質之出菇試驗

將上述滅過菌之不同稻草與木屑混合比例之太空包移入冷卻室隔夜冷卻後，以人工定量接種前述秀珍菇生長速度最快和最慢之二品系之木屑菌種，接種後太空包移入培養室定溫 (24 ± 1℃) 培養 60 天，菌絲生長期間不照光。之後去除太空包頸部棉塞並移入出菇室，溫度設定白天為 26℃、夜間為 18℃，利用超音波加濕機設定溼度為 95%，二氧化碳濃度為 1000 ppm，以刺激原基體形成，小

表 1. 稻草和木屑不同混合比例 (乾重) 基質之含水量與酸鹼值測定

Table 1. Water content and pH in substrates with different ratio of rice straw and sawdust

Ratio of rice straw and sawdust (w/w) in substrate [z]	Water content (%) [y]	pH [y]
1：0	62.6	6.6
3：1	62.0	6.5
1：1	61.2	6.6
1：3	61.1	6.4
0：1	62.6	6.3

[z] All the substrates were amended with 20% rice bran, 25% wheat bran and 1% $CaCO_3$ by dry weight basis.

[y] Autoclaved substrates were measured for water content using a moisture analyzer and for pH value using a pH meter.

菇體密集產生後將溫度設定至 26℃，控制濕度於 85–90% 間，二氧化碳濃度提高設定為 1500 ppm 使菇柄抽長，待菇傘直徑發育至 4–5 cm 大小左右採收，調查各處理之產量、生物效率及生物轉化率。生物效率 (%) 之計算方程式：採收菇體之鮮重/接種時之栽培基質乾重 × 100。生物轉化率 (%) 之計算方程式：採收菇體之乾重/接種時之栽培基質乾重 × 100。每 12 包為 1 重複，每一處理 6 重複，試驗採逢機排列，於同一環控栽培室內循常規管理，採收 3 週期，對產量、生物效率及生物轉化率以 SAS 統計分析軟體進行變方分析 (analysis of variance, ANOVA) 後，以最小顯著差異性測驗 (least significant difference test, LSD test) 探討各處理間平均值之差異性。

生產收益評估

目前市場木屑價格為每公斤 3.2 元，其含水量約為 55%，乾稻草每公斤售價 4 元，含水量約為 10%，米糠和粉頭的平均價格分別為每公斤 8.5 元和 9.0 元，兩者之含水量均約為 10%，碳酸鈣每公斤 6.0 元，秀珍菇每公斤批發價格為 65 元，分別計算不同稻草與木屑混合比例之成本與收益，人工成本與電力等其他成本每包約為 2 元，扣除後計算不同木屑與稻草混合比例栽培秀珍菇之效益。

結　　果

比較不同菌株菌絲生長速度

為了解本試驗所收集之秀珍菇菌株之生長差異，因此測試不同菌株於 PDA 培養基上之菌絲生長速度，結果顯示，在 26℃ 培養溫度下生長最快的菌株為 SJ-10，菌落大小為 4.9 cm ± 0.5 cm，其次為 SJ-06、SJ-05、SJ-12、SJ-09，而以 SJ-11 菌株為最慢，菌落大小為 3.4 cm ± 0.7 cm (圖 1)。為了評估不同秀珍菇菌株對於稻草之利用性，選取生長速度最快的 SJ-10 和最慢的 SJ-11 二菌株做為後續試驗之用。

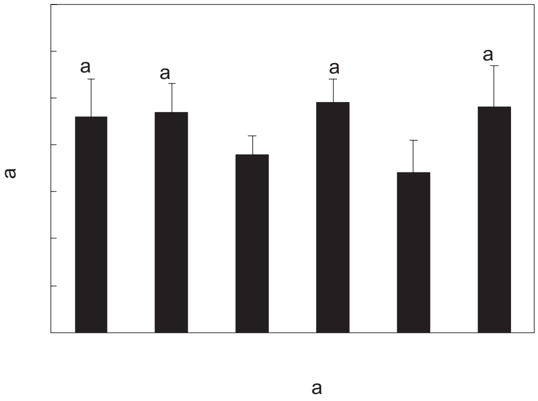

圖 1. 比較秀珍菇不同菌株在馬鈴薯葡萄糖洋菜培養基之菌絲生長情形。

Fig. 1. Comparison of mycelial growth of six strains of *Pleurotus sajor-caju* on potato dextrose agar at 26℃ for 6 days. Error bar is the standard error of mean ($n = 6$).

不同稻草和木屑混合比例對不同秀珍菇菌株之菌絲生長、產量、生物效率和生物轉化率的比較

SJ-10 和 SJ-11 兩菌株生長在不同比例的稻草與木屑混合基質中，菌絲生長、產量、生物效率和生物轉化率之變方分析結果顯示所有特性在菌株與不同混合比例基質之交感效應皆呈極顯著 ($P < 0.01$) (表 2)，表示不同菌株會隨著不同比例配方而有差異表現。在菌絲生長方面，兩菌株均以培養在稻草與木屑混合比例 1：3 之基質和全木屑處理者之菌絲生長最快，而菌株 SJ-10 以在稻草與木屑混合比例 3：1 生長最慢，但 SJ-11 之菌絲則以在稻草與木屑混合1：1之基質者生長最慢 (表 3)。

進一步比較兩菌株栽培於不同比例基質中的出菇表現 (表 3)，結果發現 SJ-10 菌株生長在稻草與木屑混合比例 1：1 之基質的產量、生物效率和生物轉化率最高，和稻草與木屑混合比例 1：3 之處理在產量及生物效率雖無顯著性差異，但生物轉化率以 1：1 處理者顯著高於 1：3 之處理。SJ-11 菌株培養在稻草與

表 2. 秀珍菇菌株和不同稻草、木屑混合比例基質對菌絲生長、產量、生物效率和生物轉化率之變方分析

Table 2. ANOVA for strain and rice straw and sawdust ratio in the substrates on colony size, yield, biological efficiency and biological conversion rate of *Pleurotus sajor-caju*

		Mean square			
Source [z]	DF	Colony size	Yield	Biological efficiency	Biological conversion rate
S	1	1.77*	1148.44**	101.47**	5.74**
R	4	3.03**	6561.40**	579.74**	3.82**
S × R	4	1.50**	3779.52**	333.94**	4.34**
Error	50	0.34	145.16	12.83	0.25

[z] S, strain of *Pleurotus sajor-caju*; R, ratio of rice straw and sawdust in substrate.
*, ** Significant at 5% and 1% levels, respectively.

表 3. 秀珍菇菌株 SJ-10 和 SJ-11 在不同稻草、木屑混合比例 (乾重) 基質之菌絲生長、產量、生物效率和生物轉化率分析

Table 3. Effect of rice straw and sawdust ratio in substrates on colony size, yield, biological efficiency and biological conversion rate of two strains of *Pleurotus sajor-caju*

Ratio of straw and sawdust (w/w) in substrate [z]	Colony size (cm)		Yield (g/bag)		Biological efficiency (%)		Biological conversion rate (%)	
	SJ-10	SJ-11	SJ-10	SJ-11	SJ-10	SJ-11	SJ-10	SJ-11
1：0	8.9 ± 0.3 b [y]	8.4 ± 0.8 bc	164.1 ± 11.3 c	115.8 ± 16.6 d	48.8 ± 3.4 c	34.4 ± 4.9 d	6.7 ± 0.4 b	4.3 ± 0.6 c
3：1	8.2 ± 0.8 c	9.0 ± 0.6 ab	174.2 ± 7.2 bc	150.3 ± 15.3 c	51.8 ± 2.2 bc	44.7 ± 4.6 c	6.5 ± 0.5 b	5.9 ± 0.5 b
1：1	8.9 ± 0.6 b	7.8 ± 0.5 c	192.3 ± 13.3 a	166.4 ± 14.5 c	57.2 ± 4.0 a	49.5 ± 4.3 c	7.5 ± 0.3 a	5.9 ± 0.6 b
1：3	9.6 ± 0.4 a	9.2 ± 0.5 a	181.7 ± 11.4 ab	222.5 ± 11.1 a	54.0 ± 3.4 ab	66.1 ± 3.3 a	6.7 ± 0.4 b	7.8 ± 0.4 a
0：1	9.6 ± 0.4 a	9.2 ± 0.7 a	175.8 ± 6.0 bc	189.4 ± 9.2 b	52.2 ± 1.8 bc	56.3 ± 2.7 b	5.8 ± 0.2 c	6.3 ± 0.3 b

[z] All the substrates were amended with 20% rice bran, 25% wheat bran and 1% $CaCO_3$ by dry weight basis.
[y] Values are mean ± standard error. Means in each column followed by the same letter(s) are not significantly different at 5% level by LSD test.

木屑混合比例 1：3 之處理，無論在產量、生物效率和生物轉化率均顯著高於全木屑和其他不同比例混合之處理。SJ-10 菌株培養在稻草與木屑混合比例 1：1 之處理的平均每包產量、生物效率和生物轉化率分別為 192.3 g、57.2% 和 7.5%，而 SJ-11 菌株培養在稻草與木屑混合比例 1：3 之處理的平均每包產量、生物效率和生物轉化率分別為 222.5 g、66.1% 和 7.8%。SJ-10 菌株以 100% 稻草栽培下的產量和生物效率最低，但和稻草與木屑混合比例 3：1 及全木屑處理者無顯著差異，而 SJ-11 菌株以 100% 稻草栽培的產量、生物效率及生物轉化率皆最低。

不同稻草和木屑混合比例對秀珍菇成本收益比較

以目前市場木屑價格為每公斤 3.2 元，然其含水量約為 50–55%，因此若換算成乾木屑之價格每公斤約需 6–7 元、乾稻草每公斤售價約為 4 元，每壹萬包的木屑太空包成本為 29,600 元 (表 4)，隨著稻草添加比例的增加，太空包的成本隨之降低。以秀珍菇每公斤批發價格 65 元計算，栽培 SJ-10 品系以稻草取代 50% 木屑的所得為 125,021 元，扣掉每包的人力和水電成本約為 2 元，以及材料費 25,400 元，淨利約為 80,000 元/萬包，比以全木屑種植者多出約 15,000 元/萬包。如果以 SJ-11 品系栽培在稻草取代 25% 木屑之處理，扣除材

表 4. 秀珍菇菌株培養在不同稻草、木屑混合比例 (乾重) 基質每萬包成本收益比較
Table 4. Comparison of production costs and net profits of the two strains of *Pleurotus sajor-caju* grown in substrates containing different ratio of rice straw and sawdust

Ratio of straw and sawdust (w/w) in substrate [z]	Cost (NT$) [y]	Yields (kg)		Income (NT$) [x]		Net profit (NT$) [w]	
		SJ-10	SJ-11	SJ-10	SJ-11	SJ-10	SJ-11
1：0	22,900	1640.8	1157.9	106,652.0	75,263.5	63,752.0	32,363.5
3：1	24,400	1742.2	1503.1	113,243.0	97,701.5	68,843.0	53,301.5
1：1	25,400	1923.4	1663.9	125,021.0	108,153.5	79,621.0	62,753.5
1：3	28,500	1817.3	2224.5	118,124.5	144,592.5	69,624.5	96,092.5
0：1	29,600	1757.7	1894.4	114,250.5	123,136.0	64,650.5	73,536.0

[z] All the substrates were amended with 20% rice bran, 25% wheat bran and 1% $CaCO_3$ by dry weight basis.
[y] Cost per 10000 bags.
[x] Fresh mushroom was calculated as NT$ 65/kg.
[w] Profit per 10000 bags.

料成本及基本支出，比以全木屑種植者多出約 22,500 元/萬包。

討　論

世界各國極力發展菇類產業，除因其本身具有豐富之食用與藥用價值外，更由於菇類可利用農業廢棄物進行栽培 (Poppe 2004; Iqbal *et al*. 2005)，不僅可以較低廉的價格甚至免費取得生產所需之原料，更可對農業廢棄物進行再利用，進而減少環境污染 (Ragunathan *et al*. 1996; Stamets 2000; Poppe 2004)。菇類栽培的收入常常高於種植其他的農作物，尤其是水稻，使得許多國家之政府願意設立專屬的研究機構扶植菇類產業，例如越南、韓國與中國。在所有的菇類栽培中又以蠔菇類能利用的農業廢棄物種類最為廣泛，包括香蕉假莖與葉、咖啡渣、油菜莖、大豆莖、花生殼、高粱桔稈、甘蔗渣和稻草等 (Poppe 2004)，在許多國家，只將這些材料先以巴士德滅菌 (Pasteurization) 處理 (Murugesan *et al*. 1995; Royse 2003) 或其他簡易方式消毒後 (Basak *et al*. 1996; Shashirekha *et al*. 2002; Nageswaran *et al*. 2003)，再以手工方式裝填至塑膠袋中，也因此在許多國外報告中並未提及需要對供試材料進行裁切。國內菇類產業長久下來已形成固定的產業鏈，對於機器化操作模式有較高依賴程度，且有專門的太空包製作廠商可將所有的基質裝填入塑膠袋中，經過滅菌後並接入菌種，方才販售給菇農進行栽培，然國內許多木屑替代基質之研究中，發現材料顆粒若過大或纖維過長，無法有效利用現行機械設備進行太空包之製作，導致無法實際應用於產業上。

本研究利用切草機將稻草切成約 1 cm 之長度，配合現行機械化太空包製作模式，所製成之太空包，不僅可成功栽培秀珍菇，且可有效提升秀珍菇之產量。結果顯示以 SJ-10 菌株培養在稻草與木屑 1：1 混合之基質時生物效率可達 57.2%，而 SJ-11 菌株則以木屑與稻草 3：1 混合之基質栽培之生物效率高達 66.1%，均顯著高於以全木屑栽培者，此結果與 Liang *et al*. (2011) 以不同替代資材進行秀珍菇栽培試驗之結果相異，該研究指出以稻草替代 1/3 木屑的生物效率 27.29%，為所有稻草替代比例中生物效率最高，但仍顯著低於全木屑處理者之生物效率 39.55%，究其造成差異的原因，除所使用的菌株不同之外，可能是與稻草的顆粒大小有關。此外，Zhang *et al*. (2002) 曾針對稻草和麥稈之利用型式對秀

珍菇產量之影響試驗，顯示稻草以長 2.5 cm 且經輾碎過的型式種植秀珍菇效果最佳，其可能之原因在於稻草的長度如果過長，可能使秀珍菇菌絲無法快速分解利用，而導致產能低落。但當粉碎稻草之長度只有 0.5 cm 時，卻會因為片段太小使得袋中基質過度緊實 (over-compaction)，進而阻礙了基質空隙空間與袋口空隙的氣體交換，反而導致產量減少的狀況。

除了稻草的長度之外，秀珍菇菌株間對於稻草與木屑的混合比例亦有不同的適應性，SJ-11 菌株在以稻草替代 25% 木屑的處理產量最高，而 SJ-10 菌株則以稻草和木屑 1：1 混合的產量最好，SJ-10 菌株對稻草的適應性較高，即使稻草與木屑以 3：1 混合，其產量和使用全木屑對照組未達顯著性差異，但 SJ-11 菌株僅有稻草取代 25% 木屑之處理明顯高於全木屑處理者。由於不同的菌株生長速度不同，SJ-10 菌株在 PDA 上的生長速度明顯較 SJ-11 菌株快，對於以稻草為基質的分解與利用能力也可能存在差異，因而利用稻草取代部分木屑時，菌株的篩選相當重要 (Chen 2005)。整體而言，不同菌株在不同配方所得生物效率和生物轉化率大致上是與產量呈正相關，惟其產生子實體之甜度和硬度經測量後，在不同比例稻草與木屑的混合基質之間無顯著差異性 (data not shown)。

就栽培之收益而言，SJ-10 菌株利用稻草取代部份的木屑，即使取代了 75% 木屑後的產量和全木屑對照組相近，然而因為乾稻草購入的成本比木屑便宜許多，整體收益仍可比對照組全木屑處理者每包多出約 0.4 元。而 SJ-11 菌株的收益概算僅有取代 25% 木屑的配方優於對照組，在材料成本上，稻草替代 25% 木屑可以比全木屑對照組相對少了 0.11 元/包，再加上產量相對增加，因而使得所得淨利扣除成本後可比全木屑之對照組多出約 2.2 元/包的利潤。

國內所使用的秀珍菇栽培菌株可能經過長時間的馴化，對於木屑的適應性良好，未來如果要增加稻草取代木屑的比例，甚至和國外同步完全以稻草栽培，就必須進行菌株選育。由本研究之結果顯示，只要選用適當的秀珍菇菌株，可以讓稻草替代 25–50% 的木屑後，應用於秀珍菇之生產，不僅可降低原物料成本且能增加秀珍菇之產量和收益，同時還可減少約 4500–9000 t 稻草被露天燃燒，並能每年降低約 9000–18,000 t 木屑之使用，因此利用稻草替代木屑，不僅可以減少森林林木的砍伐和增加農業廢棄物的再利用，更有利於秀珍菇產業之發展，達到發展菇類產業與保護環境的雙重目的。

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Rice Straw for Production of Phoenix-tail mushroom, *Pleurotus sajor-caju*[1]

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Abstract

Li, W. S., Y. S. Lue, and M. H. Chen. 2012. Rice straw for production of Phoenix-tail mushroom, *Pleurotus sajor-caju*. J. Taiwan Agric. Res. 61:90–99.

Phoenix-tail mushroom [*Pleurotus sajor-caju* (Fr.) Singer] is one of the five most economically important mushrooms produced in Taiwan. Sawdust from trees is a major ingredient in the substrate for the production of this mushroom, with more than 30,000 tons used for its production annually. Using sawdust for mushroom production is not only expensive but also harmful to the environment. Since rice straw is a major agricultural waste in Taiwan, this study was conducted to determine the possibility of replacing wood sawdust with rice straw for production of Phoenix-tail mushroom. The experiment was set up with five treatments, which contained a mixture of rice straw and sawdust at 1:0, 3:1, 1:1, 1:3 and 0:1 ratio (by dry weight). Six strains of *P. sajor-caju* were tested for mycelial growth on potato dextrose agar and the fastest-growing strain SJ-10 and the slowest-growing strain SJ-11 were selected for production of fruiting bodies in different treatments. Results showed that the most suitable substrates for mycelial growth of strains SJ-10 and SJ-11 were the substrates containing rice straw-sawdust mixture at a ratio of 1:3 and 0:1 by dry weight. The strain SJ-10 produced highest yield (192.3 grams of fruiting bodies per bag) for the treatment of rice straw-sawdust mixture at 1:1 ratio, whereas the strain SJ-11 produced highest yield (222.5 grmas of fruiting bodies per bag) for the treatment of rice straw-sawdust mixture at 1:3 ratio. Compared to the treatment of substrate containing 100 % sawdust without rice straw, the net profit of each bag was increased by 1.5 dollars (NT$) for the strain SJ-10 grown in the substrate containing rice straw-sawdust mixture at 1:1 ratio and 2.25 dollars (NT$) for the strain SJ-11 grown in the substrate containing rice straw-sawdust mixture at 1:3 ratio. These results suggest that rice straw has potential for replacing sawdust for commercial production of Phoenix-tail mushroom and the suitable amount of rice straw in the substrate varies with strains of *P. sajor-caju*.

Key words: *Pleurotus sajor-caju*, Rice straw, Yield, Biological efficiency, Biological conversion rate.

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台灣農業研究 (J. Taiwan Agric. Res.) 61(2):100–111 (2012)

氮源型態及濃度對丹參毛狀根生長與丹參酮產量之影響[1]

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摘 要

詹効松、羅子庭、陳威臣、夏奇鈮、蔡新聲。2012。氮源型態及濃度對丹參毛狀根生長與丹參酮產量之影響。台灣農業研究 61:100–111。

利用具有快速生長特性之轉殖毛狀根做為培養材料是組織培養大量生產植物二次代謝物之新趨勢，本研究以 B5 鹽類配方作為丹參 (*Salvia miltiorrhiza*) 毛狀根之基礎培養基，探討 B5 培養基中兩種型態之氮源–硝酸鉀 (KNO_3) 與硫酸銨 [$(NH_4)_2SO_4$)] 及其濃度對於丹參毛狀根生質量、丹參酮含量及其產量之影響。將 B5 鹽類配方中之硝酸鉀濃度分別調整為 0–49.5 mM，B5 配方中之其餘成分及濃度維持不變，毛狀根經 8 週培養後，顯示以 12.4–37.1 mM 硝酸鉀處理具有較高之生質量；丹參酮的含量及產量則以 37.1 mM 硝酸鉀處理最高，為對照組 B5 培養基 (24.7 mM 硝酸鉀) 的 2 倍。將 B5 鹽類配方中之硫酸銨濃度分別調整為 0–2.0 mM 進行毛狀根培養，B5 配方中之其餘成分及濃度維持不變，結果顯示毛狀根於不含硫酸銨之培養基中可獲得最高之生質量 10.25 g/L；在丹參酮的含量及產量方面，則以 0.5 mM 硫酸銨處理最高，分別為對照組 B5 培養基 (1.0 mM 硫酸銨) 的 2.8 倍及 4.1 倍。因培養結果顯示硫酸銨對毛狀根的生長具抑制作用，因此採用二階段培養方法，亦即將丹參毛狀根先於不含硫酸銨的 B5 培養基中培養 4 週後，再以饋料方式添加硫酸銨於培養液中，使其濃度為 1.0 mM 及 2.0 mM 並繼續再培養 4 週，結果顯示在培養 4 週後以饋料添加 1.0 mM 硫酸銨培養方式，相較全程 8 週培養於初始濃度為 1.0 mM 硫酸銨之 B5 培養基之對照組，在毛狀根生質量方面可提高 1.3 倍，在總丹參酮產量方面則提高 2.2 倍。以上結果顯示，氮源型態及濃度對丹參毛狀根生長與丹參酮產量有明顯之影響，提高 B5 鹽類配方中之硝酸鉀濃度、降低硫酸銨濃度、或以二階段培養方式將毛狀根於不含硫酸銨之培養基中培養，生長後期利用饋料添加硫酸銨，皆能促進毛狀根生長，並增加丹參酮的累積，是現階段較佳之培養策略。

關鍵詞：丹參、毛狀根、丹參酮、氮源、二階段培養。

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前　言

植物是人類依賴之重要自然資源，除提供人類糧食所需之外，由植物體中產生的二次代謝物 (secondary metabolites) 更可作為食品、染料、香料與藥物之用，許多植物的二次代謝產物因成分複雜不易以化學方法合成，至今仍以從植物體中提取為主，但這些二次代謝物在植物體內的含量往往偏低，增加了原料取得的成本。以組織培養技術大規模培養植物之細胞、器官乃至於植物體的培養方式已有相當之基礎，應可作為大規模生產特定植物二次代謝物之替代方案，不但可降低原料的取得成本，更可抒解因為過度採擷造成自然資源逐漸匱乏的窘境 (Kim *et al*. 2002a)。

丹參 (*Salvia miltiorrhiza*) 為唇形科 (Lamiaceae) 鼠尾草屬多年生草本植物，在傳統醫學治療上主要作為活血化瘀之用，近代西方醫學之研究亦證實丹參具有抑制血小板凝集的能力 (Hur *et al*. 2005)，對於冠狀動脈有擴張作用，並可作為抗心絞痛的藥物 (Lei & Chiou 1986)。一般而言由丹參中萃取出的化學成分包含脂溶性的二萜醌類和水溶性酚酸類，其中脂溶性成分有丹參酮 I (tanshinone I)、丹參酮 IIA (tanshinone IIA)、丹參酮 IIB (tanshinone IIB)、隱丹參酮 (cryptotanshinone) 等 (Takahashi *et al*. 2002)，這些脂溶性化合物具有高度的抗氧化活性及強大的自由基清除能力 (Wang *et al*. 2003)。在丹參酮的藥理研究中，Kang *et al*. (2000) 指出，丹參酮 I 與隱丹參酮能夠抑制 interleukin-12 (IL-12) 和 interferon-gamma (IFN-γ) 的產生，具有抗發炎與減緩免疫反應產生；Yuan *et al*. (2003) 指出丹參酮 IIA 具抗腫瘤活性，對急性骨髓型血癌 (acute promyelocytic leukemia) 細胞能產生誘導分化作用，使癌細胞分化為正常細胞，此外也能誘導人類鼻咽癌細胞與人類肺腺癌細胞等多種癌細胞走向細胞凋亡 (apoptosis)；隱丹參酮除了被認為對革蘭氏陽性菌具有抗菌能力 (Lee *et al*. 1999) 外，並可抑制基質金屬蛋白酶 (matrix metalloproteinases)，具有抗血管內膜增生和抗動脈粥狀硬化等效用，極具臨床治療應用之潛力 (Suh *et al*. 2006)。

目前利用植物組織培養技術生產植物二次代謝物，最常使用的方法有懸浮細胞培養與毛狀根培養兩種方式，其中毛狀根為藉由農桿根群菌感染植物組織後所形成具有不斷增生特性之根狀組織，相較於懸浮細胞具有生長快速、不需添加植物生長調節劑、遺傳性狀及二次代謝物生合成較為穩定等優點 (Shanks & Morgan 1999; Srivastava & Srivastava 2007)，許多植物二次代謝產物之生合成與累積亦發生於根部 (Flores *et al*. 1999)。此外利用生物性或非生物性誘引劑 (elictor)，如銀離子 (Zhang *et al*. 2004; Ge & Wu 2005a)、酵母誘引劑 (Chen *et al*. 2001)、beat-aminobutyric acid (Ge & Wu 2005b)、滲透壓逆境 (hyperosmotic stress) (Shi *et al*. 2007)、植物生長調節劑 (Gupta *et al*. 2011)，養分饋料或培養基更新 (Zhang *et al*. 2004) 等方式均證實可以增加丹參毛狀根生質量與丹參酮含量，再配合生物反應器的使用 (Kim *et al*. 2002a, 2002b)，使得毛狀根培養成為大量生產植物二代謝產物之利器。

植物體中氮源的利用途徑為硝酸態氮 (NO_3^-) 經硝酸氮還原酵素 (nitrate reductase) 還原成亞硝酸氮 (NO_2^-)，亞硝酸氮再由亞硝酸氮還原酵素 (nitrite reductase) 還原為銨態氮 (NH_4^+)，再以麩醯胺酸合成酵素 (glutamine synthetase) 將氨態氮合成為麩醯胺酸 (glutamine)，之後並轉變成其它胺基酸供植物體利用 (Layzell 1990)。硝酸態氮經由主動運輸過程進入植物體後，小部份經轉化成銨態氮型式進行代謝，大部份會以硝酸態氮型式進行儲存，即使高濃度時也不會對植物體有不良影響；而銨態

氮則經由氫離子交換後進入細胞，在細胞內經去質子化形成 NH_3，因為 NH_3 對植物毒害較大，必須先與有機酸結合形成胺基酸後再進行後續之代謝。組織培養成功的關鍵步驟之一是選擇適當的培養基，針對不同種類植物之細胞與組織所設計的培養基配方，主要亦表現在氮源種類與含量的不同，亦即藉由硝酸態氮與銨態氮兩種型態以不同濃度及比例存在於培養基中，讓不同種類植物達到最佳之生長狀態。Lin (2005) 以 MS (Murashige & Skoog 1962)、1/2 MS、WPM (Lloyd & McCown 1980)、B5 (Gamborg *et al*. 1968) 及 N6 (Chu *et al*. 1975) 鹽類配方培養基進行丹參毛狀根培養，結果發現雖然在 WPM 培養基中可得最高之毛狀根生質量，但丹參毛狀根在 B5 培養基中可得到最高之丹參酮類 (tanshinones) 含量，分析 MS、B5、WPM 與 N6 這 4 種培養基之總氮含量依序為 MS (60.03 mM/L)、N6 (35 mM/L)、B5 (27.03 mM/L) 及 WPM (16.10 mM/L)，其中 B5 培養基是為大豆根細胞培養而設計的，其總氮含量在 4 種鹽類配方中雖非最低，但其特點是含有較低的銨態氮適合根部細胞或組織的生長。Chen *et al*. (2008) 的丹參毛狀根培養研究則顯示，B5 培養液中之硝酸態氮與氨態氮濃度均會隨著培養時間增長而降低，其中又以銨態氮較硝酸態氮消耗更為快速，在毛狀根培養 3 週後即迅速減少。由此推測，培養基中提供之氮源的型態與含量的多寡，對於丹參毛狀根的生長與二次代謝產物的形成有重要的影響。

本研究以 B5 鹽類配方作為丹參毛狀根培養之基礎培養基，探討修正 B5 配方培養基中兩種形式氮源-硝酸鉀 (potassium nitrate, KNO_3) 與硫酸銨 [ammonium sulfate, $(NH_4)_2SO_4$] 的濃度對毛狀根生長與丹參酮含量與產量之影響，並進一步探討於毛狀根培養期間利用饋料添加硫酸銨之培養方式對提高丹參毛狀根生質量與丹參酮產量之可行性。

材料與方法

丹參毛狀根培養

丹參毛狀根由丹參瓶苗葉片培植體經由農桿根群菌 (*Agrobacterium rhizogenes*, R1601) 感染所得 (Lin *et al*. 2005)。毛狀根培養之固態培養基為 B5 基本鹽類配方添加 3% 蔗糖與 0.9% Bacto agar，毛狀根於 25℃ 無光照環境中進行培養，每 4 週繼代一次。液態培養之接種材料為切取於固態培養基中生長 2 週後長約 1.5 cm 之毛狀根根尖，置於裝有 20 mL 培養液的 125 mL 三角錐形瓶中，每瓶培養 5 條根尖，每處理接種 3 瓶，於 25℃ 無光照環境中利用往復式震盪培養器以 70 rpm 進行震盪培養。

丹參毛狀根乾重測定與丹參酮含量分析

將收穫後之丹參毛狀根置於烘箱以 50℃ 乾燥 48 小時或至恆重後測量其乾重，丹參酮檢品製備及 HPLC 分析依據 Chen *et al*. (2008) 方法進行。將乾燥毛狀根置入液態氮中研磨，將同一處理均質化後之毛狀根樣品粉末合併，秤取 0.5 g 樣品粉末以 10 mL 二氯甲烷 (dichloromethane)：甲醇 = 1：4 混合溶劑搭配超音波震盪器震盪 20 分鐘，並以 2700 rpm 離心 10 分鐘後取得萃取液，重複萃取 2 次，將濾液合併後以真空減壓濃縮機 (EYELA，日本) 濃縮，濃縮液經甲醇定容後以 0.45 μm 濾膜過濾，以 HPLC 法分別檢測丹參酮 I (Tanshinone I, Tan I)、丹參酮 IIA (Tanshinone IIA, Tan IIA) 與隱丹參酮 (Cryptotanshinone, Crypto)，丹參酮類標準品購自九鼎生技 (台灣)。

丹參毛狀根中總丹參酮含量與產量之計算

以樣品分析得到之隱丹參酮、丹參酮 I 與丹參酮 IIA 含量 (mg/L)，計算總丹參酮含量 (mg/g)，計算方式為：

丹參酮總含量 (mg/g) = 隱丹參酮含量 (mg/g) + 丹參酮 I 含量 (mg/g) + 丹參酮 IIA 含量 (mg/g)。

丹參酮總產量 (mg/L) = 丹參毛狀根生質量 (mg/L) × 總丹參酮含量 (mg/g)。

硝酸鉀濃度對丹參毛狀根生長及丹參酮含量及產量影響試驗

以 B5 鹽類配方作為基本培養基進行毛狀根培養，將配方中之硝酸鉀濃度分別調整為 0 mM、6.2 mM、12.4 mM、24.7 mM (B5 配方濃度，對照組)、37.1 mM、49.5 mM，配方中之其餘成分及濃度皆依照 B5 配方維持不變，每種硝酸鉀濃度培養 3 瓶，培養 8 週後調查毛狀根生質量與丹參酮含量。

硫酸銨濃度對丹參毛狀根生長及丹參酮產量影響試驗

同樣以 B5 鹽類配方為基本培養基進行毛狀根培養，將 B5 配方中硫酸銨濃度分別調整為 0 mM、0.5 mM、1.0 mM (B5 配方濃度，對照組)、1.5 mM 及 2.0 mM，配方中之其餘成分與濃度皆依照 B5 配方維持不變，每種濃度硫酸銨共培養 3 瓶，培養 8 週後調查毛狀根生質量與測量丹參酮含量。

毛狀根生長中期添加硫酸銨對丹參毛狀根生長及丹參酮產量影響試驗

丹參毛狀根在不含硫酸銨的 B5 培養液中培養 4 週後，於培養液中添加硫酸銨，使培養液中之硫酸銨濃度為 1.0 mM 及 2.0 mM，再繼續培養 4 週後調查毛狀根生質量及丹參酮含量。對照組分別為持續 8 週培養於不含硫酸銨或持續 8 週培養於含有 1.0 mM 硫酸銨之 B5 培養基之丹參毛狀根。

統計分析方法

試驗採用完全逢機設計 (Completely Randomized Design, CRD)，每處理取樣 3 瓶，分別測量毛狀根之乾重，因單瓶毛狀根之乾重取樣量過少，所以三種丹參酮含量分析是將同一處理之樣品粉末合併後再進行取樣分析。試驗所得資料經 SAS 8.2 (SAS Institute Inc. 2001) 套裝統計分析軟體進行 ANOVA 變方分析，若處理間差異顯著 ($P < 0.05$)，則利用 Least Significant Difference test (LSD) 比較各處理平均值間之差異。

結　　果

硝酸鉀濃度對丹參毛狀根生長及丹參酮含量及產量影響試驗

丹參毛狀根培養於含有 0–49.5 mM 硝酸鉀濃度的 B5 鹽類配方培養基 8 週後，結果如表 1 與圖 1 所示。培養基中硝酸鉀濃度在 0–12.4 mM 之間時，丹參毛狀根生質量會隨著硝酸鉀濃度提高而增加，以 12.4 mM 硝酸鉀試驗組之毛狀根生質量達最高，為 8.9 g/L；毛狀根生質量於 12.4–37.1 mM 硝酸鉀試驗組間並無顯著差異；但隨著硝酸鉀濃度繼續提高，毛狀根生質量有逐漸下降之趨勢。在圖 1 中可觀察到毛狀根在不含硝酸鉀之培養液中幾乎沒有生長，而隨著硝酸鉀濃度增加，可以觀察到丹參毛狀根的形態逐漸加粗。在丹參酮含量方面，除了 0 mM 硝酸鉀試驗組具有較高之隱丹參酮 (3.24 mg/g) 外，其餘各處理皆顯示毛狀根之丹參酮含量會隨培養基中硝酸鉀濃度提高而增加，其中又以隱丹參酮之含量變化最顯著；在丹參酮產量方面，以 37.1 mM 硝酸鉀濃度試驗組可得最高總丹參酮含量及產量，分別為 6.40 mg/g 與 53.42 mg/L，為原 B5 培養基處理 (24.7 mM 硝酸鉀) 的 2.1 倍和 2 倍。

硫酸銨濃度對丹參毛狀根生長及丹參酮產量影響試驗

丹參毛狀根於含有 0–2.0 mM 硫酸銨濃度的 B5 鹽類配方培養基中培養 8 週後，結果如表 2 與圖 2 所示。丹參毛狀根生質量以不含硫酸銨之試驗組最高，為 10.25 g/L，是對照組 B5 培養基 (1.0 mM 硫酸銨) 的 1.8 倍；在硫酸銨濃度為 0–1.0 mM 之間時，丹參毛狀根生質量會隨著硫酸銨濃度提高而顯著下降，當硫酸銨濃度為 1.0–2.0 mM 之間時，三試驗組之毛

表 1. 硝酸鉀濃度對丹參毛狀根生長、丹參酮含量及丹參酮總產量之影響

Table 1. Effects of potassium nitrate concentrations on biomass, tanshinone content and total tanshinone production in hairy root cultures of *Salvia miltiorrhiza*

Potassium nitrate (mM) in B5 medium [z]	Biomass (g dw/L)	Tanshinone (mg/g)				Tanshione production (mg/L)
		Tan I [y]	Tan IIA	Crypto	Total	
0.0	0.20 ± 0.06 d [x]	0.00	0.00	3.24	3.24	0.65
6.2	5.95 ± 0.20 c	0.02	0.00	0.89	0.91	5.42
12.4	8.90 ± 0.30 a	0.02	0.06	1.41	1.50	13.31
24.7	8.90 ± 0.30 a	0.20	0.53	2.35	3.08	26.15
37.1	8.33 ± 0.33 ab	0.68	1.50	4.21	6.40	53.42
49.5	7.92 ± 0.24 b	0.25	0.79	3.88	4.92	38.87

[z] B5 medium was used as basal medium except concentrations of potassium nitrate were amended. Cultures were incubated at modified B5 medium for 8 weeks and tested for biomass, tanshinone and tanshinone production.

[y] Tan I: tanshinone I; Tan IIA: tanshinone IIA; Crypto: Cryptotanshinone.

[x] Mean ± standard error (n = 3). Means followed by the same letter(s) within each column are not significantly different at 5% level according to Least Significant Difference Test.

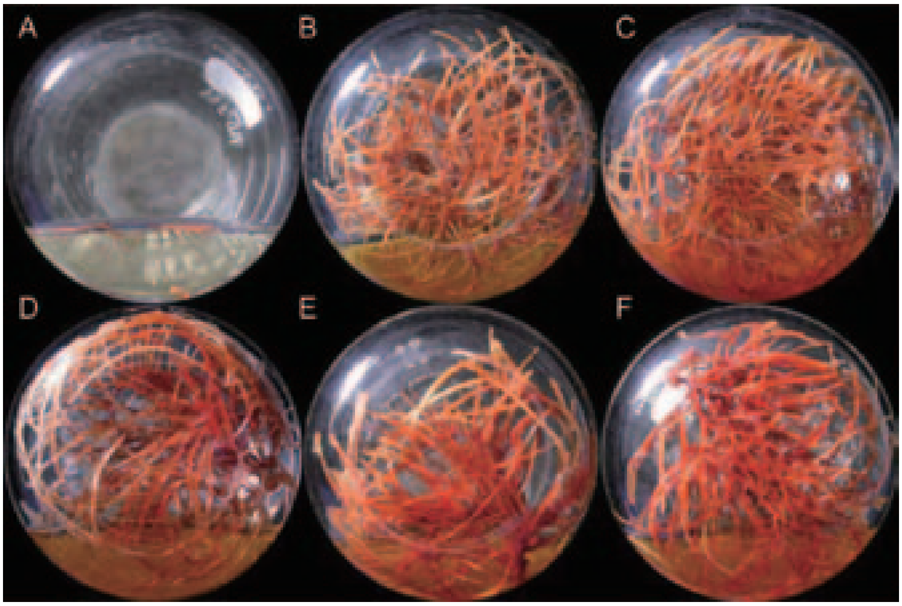

圖 1. 丹參毛狀根於不同濃度硝酸鉀之 B5 配方培養基中培養 8 週後之生長情形。

Fig. 1. Hairy roots of *Salvia miltiorrhiza* cultured for 8 weeks in the modified B5 basal medium containing various concentrations of potassium nitrate (A−F). Concentration of potassium nitrate in the medium: (A) 0 mM; (B) 6.2 mM; (C) 12.4 mM; (D) 24.7 mM; (E) 37.1 mM; (F) 49.5 mM.

狀根生質量並無顯著性差異。在丹參酮含量方面，當硫酸銨濃度為 0.5 mM 時，丹參酮含量達到最高，為 7.24 mg/g，其中丹參酮 I、丹參酮 IIA 及隱丹參酮三種丹參酮之含量分別為對照組的 2.36 倍、1.68 倍與 1.43 倍，總丹參酮產量 60.94 mg/L 為對照組的 4.1 倍。圖 2 中可觀察到硫酸銨對於毛狀根生長與丹參酮累積的影響，在硫酸銨濃度為 0 mM 的試驗組中，毛

表 2. 硫酸銨濃度對丹參毛狀根生長、丹參酮含量及丹參酮總產量之影響

Table 2. Effects of concentration of ammonium sulfate on biomass, tanshinone content and total tanshinone production in hairy root cultures of *Salvia miltiorrhiza*

Ammonium sulfate (mM) in B5 medium [z]	Biomass (g dw/L)	Tanshinone (mg/g)				Tanshinone production (mg/L)
		Tan I [y]	Tan IIA	Crypto	Total	
0.0	10.25 ± 0.62 a [x]	0.14	0.16	0.03	0.33	3.33
0.5	8.42 ± 0.33 b	3.54	1.01	2.69	7.24	60.94
1.0	5.67 ± 0.35 c	0.15	0.60	1.88	2.63	14.89
1.5	6.35 ± 0.19 c	0.40	0.58	1.48	2.46	15.59
2.0	6.20 ± 0.12 c	0.59	0.60	1.51	2.70	16.75

[z] B5 medium was used as basal medium except concentrations of ammonium sulfate were amended. Cultures were incubated at modified B5 medium for 8 weeks and tested for biomass, tanshinone and tanshinone production.
[y] Tan I: tanshinone I; Tan IIA: tanshinone IIA; Crypto: Cryptotanshinone.
[x] Mean ± standard error (n = 3). Means followed by the same letter(s) within each column are not significantly different at 5% level according to Least Significant Difference Test.

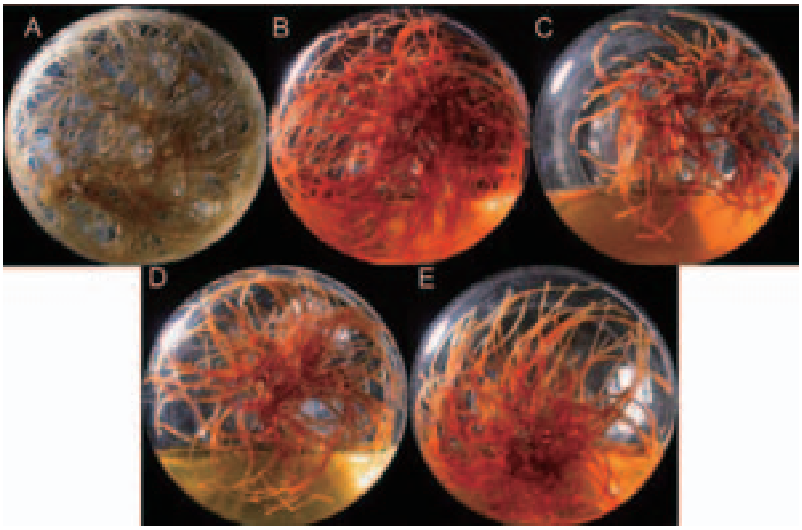

圖 2. 丹參毛狀根於不同濃度硫酸銨之 B5 配方培養基中培養 8 週後之生長情形。

Fig. 2. Hairy roots of *Salvia miltiorrhiza* cultured for 8 weeks in B5 medium containing various concentrations of ammonium sulfate (A–E). Concentration of ammonium sulfate in the medium: (A) 0 mM; (B) 0.5 mM; (C) 1.0 mM; (D) 1.5 mM; (E) 2.0 mM.

狀根生長旺盛，但毛狀根呈現淡褐色；在添加 0.5–2.0 mM 硫酸銨之試驗組中，毛狀根因累積丹參酮而呈現較深之紅色。

毛狀根生長中期添加硫酸銨對丹參毛狀根生長及丹參酮產量影響試驗

利用不含硫酸銨的 B5 培養基進行丹參毛狀根第一階段培養，在培養 4 週後添加硫酸銨使培養液中之硫酸銨濃度分別為 1.0 mM 及 2.0 mM，繼續第二階段 4 週的培養，藉由硫酸銨於培養基中出現的時間差，觀察其對丹參毛狀根生長與丹參酮累積的影響，總共 8 週培養後結果如表 3 與圖 3 所示。持續培養於完全不含硫酸銨之對照組具有最高之毛狀根生質量 11.2 g DW/L，但三種丹參酮含量皆低，總丹參酮含量僅達

表 3. 利用培養期間添加硫酸銨對丹參毛狀根生質量、丹參酮含量與總丹參酮產量的影響

Table 3. Effect of amendment of ammonium sulfate in B5 basal medium at week-4 on biomass, tanshinone content and total tanshinone production of hairy root cultures of *Salvia miltiorrhiza*

Ammonium sulfate (mM) added in culture [z]			Tanshinone (mg/g)				
Initial culture	4-week-old culture	Biomass (g dw/L)	Tan I [y]	Tan IIA	Crypto	Total	Tanshinone production (mg/L)
0.0	0.0	11.2 ± 0.50 a [x]	0.00	0.00	0.05	0.05	0.57
1.0	0.0	7.42 ± 0.26 b	0.79	1.06	2.14	3.99	29.59
0.0	1.0	9.58 ± 0.03 c	3.18	1.72	1.85	6.76	64.70
0.0	2.0	8.82 ± 0.56 c	2.66	1.62	0.66	4.94	43.53

[z] Initial and additional ammonium sulfate concentrations added at week-1 and week-4 in the modified B5 medium. Cultures were incubated at modified B5 medium for total 8 weeks and tested for biomass, tanshinone and tanshinone production.

[y] Tan I: tanshinone I; Tan IIA: tanshinone IIA; Crypto: Cryptotanshinone.

[x] Mean ± standard error ($n = 3$). Means followed by the same letter(s) within each column are not significantly different at 5% level according to Least Significant Difference Test.

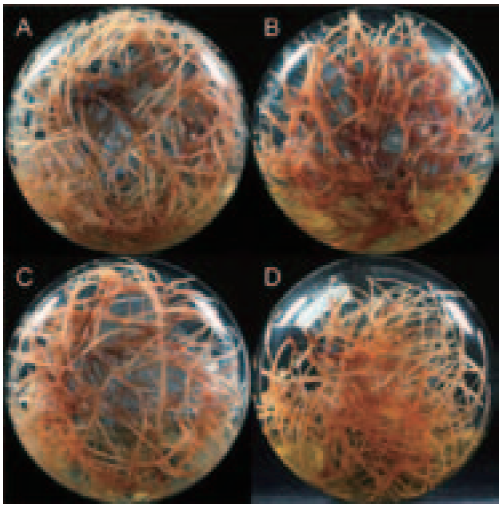

圖 3. 丹參毛狀根培養在不同濃度硫酸銨之 B5 培養基中生長 8 週之情形。培養基中硫酸銨濃度分別為 (A) 0 mM 及 (B) 1.0 mM NH_4NO_3，以及毛狀根於不含硫酸銨之培養基中培養 4 週後再添加 (C) 1.0 mM 及 (D) 2.0 mM 硫酸銨。

Fig. 3. Eight-week-old hairy root cultures of *Salvia miltiorrhiza* grown in B5 basal medium with (C−D) or without (A−B) amendment of NH_4NO_3. Time and concentration of amendment of ammonium sulfate: (A) 0 mM $(NH_4)_2SO_4$; (B) 1.0 mM $(NH_4)_2SO_4$ at week-1; (C) 1.0 mM $(NH_4)_2SO_4$; (D) 2.0 mM $(NH_4)_2SO_4$ at week-4.

0.57 mg/L；培養 4 週後添加 1.0 mM 硫酸銨之毛狀根的生長與不含硫酸銨之對照組相較顯示生長受到抑制，但其丹參酮含量與產量顯著增加，分別為不含硫酸銨對照組之 133.4 倍及 113.5 倍；與培養初始即含有 1.0 mM 硫酸銨之 B5 配方培養基之對照組相較，發現培養 4 週後再添加 1.0 mM 硫酸銨有助於毛狀根生質量與丹參酮含量的增加，生質量可以提高 1.29 倍，總丹參酮含量為 6.76 mg/g，提高 1.69 倍，丹參酮產量方面為 64.70 mg/L，提高 2.18 倍。將第二階段添加之硫酸銨濃度提高到 2.0 mM，毛狀根生質量與添加 1.0 mM 處理相較，影響並不

明顯，但總丹參酮的含量會減少，但仍較 8 週持續培養於添加 1.0 mM 硫酸銨之對照組增加 1.47 倍。

討　論

利用組織培養方式生產植物二次代謝物除了要顧及培植體的生長外，最重要的是要提高二次代謝物的產量，培養條件中作為營養供應的培養基扮演了重要的角色，尤其當培植體生質量增加與二次代謝物含量累積所需條件不一致時，如何透過對培養系統的瞭解而加以調整，研擬出一套具體可行之培養策略，是整體二次代謝物生產能否提升的關鍵。綜觀毛狀根培養所用之培養基，皆含有硝酸態氮與銨態氮兩種主要型態之氮源，前人之研究顯示硝酸態氮與銨態氮的濃度及其比例除了影響毛狀根的生長外，對於毛狀根內所含二次代謝物的產量亦有很大的影響 (Lin 2005; Chen *et al*. 2008; Cui & Murthy 2010)。Bensaddek *et al*. (2001) 在顛茄毛狀根培養的結果中顯示，硝酸鹽濃度 15.80 mM 增至 39.5 mM，不利於生物鹼含量與產量的增加，但有利於毛狀根生質量的增加；但在本研究丹參毛狀根的培養結果中則顯示，將培養基中硝酸鉀濃度從對照組 B5 配方培養基之 24.7 mM 提高至 37.1 mM，有助於總丹參酮含量與產量的提高，且毛狀根生質量的減少並不明顯，計算丹參酮 I、丹參酮 IIA 及隱丹參酮三種丹參酮含量則分別為對照組的 11 倍、2.8 倍與 1.8 倍，總丹參酮產量為對照組的 2.0 倍，其中硝酸鹽對於丹參酮 I 促進之效果最為明顯。在本研究中亦顯示，毛狀根在不含硝酸鉀培養基中無法正常生長，此點與 Lourenço *et al*. (2002) 發現紫星薊毛狀根在不含硝酸鉀培養基中幾乎沒有生長之結果相似，推測適量硝酸鹽的存在為毛狀根生長所必需。此外，Bensaddek *et al*. (2001) 在顛茄毛狀根的培養以及 Lourenço *et al*. (2002) 在紫星薊毛狀根培養的結果皆顯示，不含銨鹽的培養基有利於毛狀根生質量的增加；在本研究中丹參毛狀根培養於完全不含硫酸銨的培養基中，亦證實可得到最高之毛狀根生質量，推測銨鹽並非丹參毛狀根生長所必需，唯培養在不含硫酸銨中之丹參毛狀根其丹參酮的含量偏低，顯示硫酸銨有助於丹參酮的累積，添加少量 (0.5 mM) 之硫酸銨在丹參酮 I、丹參酮 IIA 及隱丹參酮三種丹參酮含量表現上較對照組 (1.0 mM 硫酸銨) 可提高 23.6 倍、1.7 倍與 1.4 倍，總丹參酮產量則為照組的 4.1 倍，其中硫酸銨對於丹參酮 I 促進之效果最為明顯，此點與硝酸鉀對於丹參酮 I 促進效果最佳相同。Hilton & Wilson (1995) 發現一些藥用植物毛狀根在指數生長期的第 1 天，銨態氮源即被快速用盡，在丹參毛狀根的前人研究中亦指出，銨態氮源在培養 3 週後迅速下降 (Chen *et al*. 2008)，顯示銨態氮源對於植物細胞而言是較容易吸收轉化 (同化) 之氮源，推測這也是為什麼低濃度銨鹽即可對植物細胞產生毒害的原因之一。從本研究之結果來看，銨鹽濃度對毛狀根生長及丹參酮之累積有相異之影響 (不含銨鹽可提高毛狀根之生質量，但不利丹參酮之累積)，因此硫酸銨濃度對丹參毛狀根生長及丹參酮產量之最佳濃度範圍 (0–1.0 mM 硫酸銨) 必須精確的加以界定；對於硝酸態氮源與銨態氮源之間是否具有交感作用，亦必須設計進一步之試驗才能加以釐清。

在本研究中發現移除培養基中之銨態氮源-硫酸銨，可明顯增加毛狀根生質量，但有丹參酮不累積之缺點，針對此一現象，假設在毛狀根已完成生長之階段再添加銨態氮於培養基中，推測毛狀根之生長不會受到抑制，但卻有助於丹參酮的累積，應該是較為適當之培養策略。源於上述之假設，本研究設計配合丹參毛狀根生長於培養 4 週後，以饋料添加的方式補充硫酸銨，探討在生長中後期適時調整硫酸

銨濃度含量對提高丹參酮產量之可行性。試驗結果顯示，雖然二階段饋料添加硫酸銨處理之丹參毛狀根相較於全程 8 週培養於完全不含硫酸銨之丹參毛狀根生質量仍較低，但其生質量卻明顯高於持續培養於原始濃度為 1.0 mM 硫酸銨之 B5 培養基對照組；尤其在丹參酮含量及產量表現方面，以饋料添加方式補充硫酸銨之處理明顯高於培養於不含硫酸銨以及培養於初始濃度為 1.0 mM 硫酸銨之對照組，顯示於培養中期後以饋料添加硫酸銨的培養方式，不但能減少硫酸銨影響毛狀根生長的缺點，並且能改進丹參毛狀根培養在不含硫酸銨培養基中丹參酮不累積的缺點，未來將針對提高丹參酮的產量，進一步研究二階段饋料添加硫酸銨的最適時間與最適濃度的影響。

以組織培養方式生產植物二次代謝物相較於以生產種苗為目標之培養方式複雜，因為培養系統的好壞不能單純以培植體生質量或其二次代謝物的含量高低來判斷，尤其當提高生質量與提高二次代謝物含量所需培養條件不一致時，最終必須以二次代謝物的總產量為目標來調整生產策略。本研究在丹參毛狀根生產丹參酮的培養系統中，發現氮源型態及濃度對丹參毛狀根生長與丹參酮產量有明顯之影響，其中培養基中之銨鹽對毛狀根生質產量及丹參酮產量有不同之影響，除試圖找出銨鹽之最適濃度範圍外，更發展出利用二階段饋料添加硫酸銨之方法，除可提高丹參毛狀根生質量外，並能得到較高的丹參酮產量，此一培養策略可以作為其他以組織培養生產植物二次代謝物培養系統改進之參考。

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Effect of Nitrogen Source on Biomass and Tanshinone Production of *Salvia miltiorrhiza* in Hairy Root Culture[1]

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Abstract

Chan, H. S., T. T. Lo, U. C. Chen, C. N. Hsia, and H. S. Tsay. 2012. Effect of nitrogen source on biomass and tanshinone production of *Salvia miltiorrhiza* in hairy root culture. J. Taiwan Agric. Res. 61:100−111.

There is a new tendency to produce secondary metabolites of plants in tissue culture, using fast growing hairy roots. The objective of this study was to investigate effect of potassium nitrate (KNO_3) and ammonium sulfate [$(NH_4)_2SO_4$] in the culture medium on growth of hairy roots of *Salvia miltiorrhiza* Bunge and production of tanshinone. Roots of *S. miltiorrhiza* were grown in a modified B5 medium at 70 rpm on a shaker under darkness for eight weeks and data were collected. Results showed that growth of hairy roots varied with concentrations of KNO_3 in the medium and the optimum concentration of KNO_3 was 12.4 mM, which resulted in maximum production of root biomass (8.90 g dw/L). However, the highest tanshinone content was obtained in the culture containing 37.1 mM KNO_3, representing a 2-fold increase in tanshinone production, compared to the control concentration of 24.7 mM KNO_3 in the B5 medium. When hairy roots were cultured in the B5 basal medium containing different concentrations of $(NH_4)_2SO_4$ from 0 to 2.5 mM, the maximum production of root biomass (10.25 g dw/L) was the B5 medium without $(NH_4)_2SO_4$. Although growth of hairy roots was inhibited by $(NH_4)_2SO_4$ at all the concentrations (0.5−2.5 mM) tested, an increase in tanshinone contents was observed in the treatment of $(NH_4)_2SO_4$ at lower concentration of 0.5 mM. The tanshinone production in the treatment of 0.5 mM $(NH_4)_2SO_4$ was 4.1-fold higher than the treatment of 1.0 mM $(NH_4)_2SO_4$ representing the control concentration of $(NH_4)_2SO_4$ in the B5 medium. Since root growth was found inhibited by $(NH_4)_2SO_4$ in the B5 medium, in subsequent experiment hairy roots were grown in the B5 medium without $(NH_4)_2SO_4$ for 4 weeks before adding 1.0 mM or 2.0 mM $(NH_4)_2SO_4$ for another 4 weeks culturing. This two-stage model [weeks 1−4 without $(NH_4)_2SO_4$ and weeks 5−8 with 1.0 mM $(NH_4)_2SO_4$] increased root biomass of *S. miltiorrhiza* by 1.3 fold and tanshinone production by 2.2 fold, compared to the control treatment of 1.0 mM $(NH_4)_2SO_4$ in the B5 medium for the entire culturing period of 8 weeks. In conclusion, biomass and tanshinone production in hairy root culture of *S. miltiorrhiza* were

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affected significantly by nitrogen sources in the B5 medium. Increasing concentration of KNO_3, reducing concentration of $(NH_4)_2SO_4$ in the B5 medium or using the two-stage model of application of $(NH_4)_2SO_4$ is useful in enhancing biomass as well as tanshinone production in hairy root culture of *Salvia miltiorrhiza*.

Key words: *Salvia miltiorrhiza*, Hairy root, Tanshinone, Nitrogen sources, Two-stage feeding model.

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誘引劑對高氏柴胡懸浮細胞生長與柴胡皂苷含量累積之影響[1]

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摘　要

陳威臣、夏奇鈮、葉茂生、曹進義、蔡新聲。2012。誘引劑對高氏柴胡懸浮細胞生長與柴胡皂苷含量累積之影響。台灣農業研究 61:112–123。

高氏柴胡 (*Bupleurum kaoi* Liu, Chao et Chuang) 懸浮細胞於含有 0.2 mg/L 二氯苯氧基乙酸 (2,4-dichlorophenoxyacetic acid, 2,4-D) 之 1/2 MS 液態培養基中於低光照下以 100 rpm 轉速培養，細胞之生長在接種後 0–2 週為緩慢生長期，在 2–4 週呈現快速生長期，在培養 4 週後進入生長靜止期。振盪速度試驗結果顯示，80 rpm 較 100 rpm 振盪速率在培養 4 週後，具有較高之細胞生長量。細胞接種密度試驗結果顯示，以 12% 密度接種之細胞生長最佳，接種密度較高 (24%) 或較低 (6%) 的細胞生長表現均較差，但同樣接種密度 (12%) 下，以較大培養容器為佳 (125-mL vs. 250-mL 錐形瓶)。添加誘引劑之試驗結果顯示，在處理 2 週後，所有誘引劑處理之細胞鮮重皆受到顯著抑制；但在細胞乾重方面，酵母抽出物處理與對照組之乾重相較並無顯著差異，僅茉莉酸與高濃度離層酸處理對細胞乾重有顯著抑制之效果，但三種誘引劑處理對懸浮細胞之柴胡皂苷 (saikosaponin a 及 saikosaponin d) 累積均有促進效果，柴胡皂苷含量可提高 1.9–2.0 倍，產量可提高 1.6–1.8 倍。進一步施用 500 mg/L 酵母抽出物作為誘引劑，結果顯示施用 2 週後柴胡皂苷含量較對照組增加 2.1 倍，而柴胡皂苷產量可達對照組之 2.8 倍，顯示在細胞生長後期施用誘引劑並在特定反應時間後收穫細胞，有助於二次代謝產物柴胡皂苷之產生與累積。

關鍵詞：高氏柴胡、藥用植物、懸浮細胞培養、柴胡皂苷、誘引劑。

前　言

柴胡 (Chai-Hu, Radix Bupleuri) 為重要之傳統中藥材，中國藥典所列正品柴胡為柴胡屬 (*Bupleurum*) 之北柴胡 (*Bupleurum chinense*) 與南柴胡 (*Bupleurum scorzonerifolium*)，日本則以三島柴胡 (*Bupleurum falcatum*) 為正品 (Gan 1985; Pan *et al*. 2002)，台灣特有種高氏柴胡 (*Bupleurum kaoi*) 之柴胡皂苷含量及保肝效果

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已被證實優於北柴胡與三島柴胡 (Chen *et al*. 2005; Yen *et al*. 2005)。柴胡根部所含之柴胡皂苷 a 與 d (saikosaponin a, d; SSa, SSd) 為具有藥理生物活性的主要物質，近來許多團隊積極針對南柴胡與高氏柴胡之醫療機制加以研究，證實具有優異的保肝、抗 B 型肝炎病毒、抗氧化、消炎、促進腸蠕動與抑制癌細胞增生效果，在現代醫藥學的重要性與日俱增 (Hsu *et al*. 2004; Wang *et al*. 2004; Cheng *et al*. 2005)。

藥用植物的開發與利用是植物與藥學專家的共同努力目標，在合成藥當道的今日，仍有許多藥物須由傳統植物萃取而來；近年來許多野生藥用植物生長環境遭受破壞已瀕臨滅種；且有鑑於許多藥用植物需特殊環境栽培，或因生育緩慢而無法大量取得，這些因素讓利用離體細胞或器官培養生產二次代謝物成為最佳替代方式 (Nalawade *et al*. 2003; Tripathi & Tripathi 2003; Mulabagal *et al*. 2004)；例如利用細胞培養方式生產紫杉醇 (taxol) (Chang *et al*. 2004) 與喜樹鹼 (camptothecine) (Fulzele *et al*. 2001) 等重要藥物成分，亦已證實此一模式用於商業生產的可行性。

就懸浮細胞培養增殖效率而言，影響因子除培養基組成分外，振盪速度 (Chueh *et al*. 2000) 與細胞接種密度 (Chang *et al*. 2004) 也是影響之重要因子。Radman *et al*. (2003) 指出，細胞培養時添加誘引劑 (elicitors) 可提高培養細胞之二次代謝物含量。前人研究結果顯示，YE 處理可促進丹參酮 (tanshinones) (Chen & Chen 2000)、人參皂苷 (ginsenoside) (Lu *et al*. 2000) 與紫杉醇 (Wang *et al*. 2001) 含量；Li *et al*. (2003) 指出，添加酵母抽出物 (yeast extract, YE) 之處理則可能導致離層酸 (abscisic acid, ABA) 含量增加，因而促進丹參酮累積。添加茉莉酸 (jasmonic acid, JA) 或甲基茉莉酸 (Methyl Jasmonate, MJ) 則可增加人參皂苷 (Lu *et al*. 2000)、青蒿素 (artemisinin) (Baldi & Dixit 2008) 與二氫血根鹼 (dihydrosanguinarine) (Cho *et al*. 2008) 的含量。然而，亦有報告指出施用誘引劑會導致細胞生長受到抑制 (Chen & Chen 2000; Li *et al*. 2003)。Chen *et al*. (2005, 2008) 的研究結果顯示，高氏柴胡癒合組織培養 8 週後之柴胡皂苷 SSa 與 SSd 加總含量達約市售品 (北柴胡乾燥根) 的 9%；如何優化此一懸浮細胞培養系統以提高柴胡皂苷的生產，值得吾人進一步研究。本研究首先建立高式柴胡懸浮細胞培養之生長曲線，用以瞭解細胞隨著時間增加的生長量變化，並針對振盪速率與細胞接種密度優化懸浮細胞生長，而後並藉由添加 YE、JA 與 ABA 等誘引劑提高柴胡皂苷產量，逐步建立高氏柴胡細胞懸浮培養生產柴胡皂苷之生產系統。

材料與方法

供試材料與培養條件

取高氏柴胡 (*Bupleurum kaoi*) 瓶苗葉片培養於 1/2 MS (Murashige & Skoog 1962) 基本鹽類添加 0.2 mg/L 2,4-D 及 3% 蔗糖之培養基 (D2 培養基) 以誘導癒傷組織；取 0.5 g 癒合組織切碎後，移入內含 20 mL D2 培養基之 125-mL 錐形瓶中，以 100 rpm 轉速振盪培養，培養基每 3 週更換一次，經 5–6 次繼代培養後，利用篩網 (0.84 mm) 過篩取得大小一致之懸浮細胞作為後續試驗的材料。培養環境為 25 ± 1℃ 低光照 (10 $\mu mol/m^2/s$)，培養之錐形瓶置於水平迴轉振盪器 (Orbital Shaker, SK-302AB, SanKuan, Taiwan) 以 80 或 100 rpm 轉速進行培養 (Chen *et al*. 2005)。

懸浮培養細胞之生長曲線調查

取 1 mL 沉降體積 (packed cell volume, PCV) 細胞接種於含 24 mL D2 液態培養基之 125-mL 有臂錐形瓶 (總體積為 25 mL)，以 100 rpm 振盪速率進行培養，共培養 25 支有臂錐形瓶，每週利用錐形瓶之側臂調查懸浮細胞之 PCV

值，共培養 5 週。以培養時間為橫座標，PCV 值為縱座標，繪出細胞之生長曲線。

振盪速率與細胞接種密度對懸浮培養細胞生長增殖之影響

將 3 mL PCV 細胞接種於含 22 mL D2 液態培養基之 125-mL 有臂錐形瓶 (總體積為25 mL)，分別以 80 與 100 rpm 振盪速度培養，每 2 天調查一次 PCV 值至第 28 天。細胞接種密度試驗係利用 3 mL PCV 細胞接種於 125-mL 與 250-mL 錐形瓶，培養總體積在兩種錐形瓶中分別為 25 及 50 mL，細胞接種密度分別為 12% (3 mL/25 mL × 100%) 及 6% (3 mL/50 mL × 100%)；以同樣方式接種 6 mL PCV 細胞於兩種容積之錐形瓶中，接種密度則分別為 24% (6 mL/25 mL × 100%) 及 12% (6 mL/50 mL × 100%)，以 80 rpm 振盪速率進行細胞懸浮培養，於培養 2、3 與 4 週後調查各處理之細胞乾重。

誘引劑對懸浮培養細胞乾重與柴胡皂苷含量之影響

以 3 mL PCV 細胞接種於內含 22 mL D2 液態培養基之 125-mL 錐形瓶，以 80 rpm 振盪速率進行培養，誘引劑於細胞培養 3 週後，分別添加 1 mL YE (500, 1000 mg/L)、JA (0.119, 1.19 mM) 或 ABA (5, 50 μM) 溶液，並以添加 1 mL 無菌水與 0.2% 乙醇溶液作為對照組，再經過 1 週的培養後調查細胞乾重及其柴胡皂苷含量。

酵母抽出物處理時間對懸浮培養細胞乾重與柴胡皂苷含量之影響

以 6 mL PCV 細胞接種於內含 44 mL D2 液態培養基之 250-mL 錐形瓶 (接種密度 12%)，以 80 rpm 振盪速率進行培養，培養 3 週後分別添加 1 mL 之 500 mg/L YE 溶液與無菌水 (對照組)，再繼續培養 1 與 2 週後調查細胞乾重及其柴胡皂苷含量。

柴胡檢品之柴胡皂苷含量分析

柴胡皂苷含量測定以高壓液相層析法 (HPLC 法) 測定 (Chen *et al*. 2008)，方法簡述如下：檢品包括柴胡皂苷標準品 SSa 與 SSd，以及上述處理所得乾燥細胞樣品。標準品係利用甲醇溶解成系列濃度，進樣檢測後製成檢量線；乾燥細胞檢品磨碎後以甲醇為溶劑，經超音波震盪 30 分鐘後以濾紙過濾並收集濾液，重複萃取 3 次且合併濾液，經真空減壓濃縮後再加入甲醇定量，以 0.22 μm 濾膜過濾備用。HPLC 層析條件如下，利用氰化甲烷 (acetonitrile)：水 ＝ 40：60/50：50/40：60 (v/v) 為流動相進行定溫 (35℃) 梯度洗提分析，流速 1 mL/min，紫外線檢定波長為 203 nm，用以分析各檢品 SSa 與 SSd 含量。細胞檢品萃取液重複注射 3 次，每次進樣 10 μL 濾液，並依檢量線的線性迴歸方程式推算各檢品的 SSa 及 SSd 含量。

資料統計與分析

懸浮細胞生長曲線以 25 個有臂錐形瓶細胞 PCV 值的平均值繪製；振盪速率係調查 5 個有臂錐形瓶細胞 PCV 值的平均值；細胞接種密度與誘引劑試驗則是以每處理取樣 3 個錐形瓶進行調查。試驗均採用完全逢機設計 (completely randomized design, CRD) 設計，所得資料經 SAS 8.2 版套裝統計分析軟體進行變方分析 (analysis of variance, ANOVA)，若處理效應顯著 ($P < 0.05$)，則利用最小顯著性差異 (least significant difference, LSD) 測驗比較各處理平均值間之差異。

結　果

培養時間對懸浮培養細胞生長增殖之影響

取 1 mL PCV 之細胞培養於含有 24 mL D2 液態培養基，培養 2 週後之細胞 PCV 值約為 2.97 mL，培養 3 週之細胞 PCV 值達 6.23 mL，最大細胞生長量出現在培養 4–5 週間，其細胞

PCV 值約為 11.5 mL，但此兩週間之細胞 PCV 值並無明顯差異 (圖 1)。

振盪速率與接種細胞密度對懸浮培養細胞生長增殖之影響

將 12% 接種密度之高氏柴胡懸浮細胞分別以 80 及 100 rpm 的振盪速率培養，結果如圖 2 所示。培養前期 (0–14 天) 兩種振盪速率處理之 PCV 值並無明顯差異；培養後期 (15–28 天) 雖然以 80 rpm 振盪速率處理之懸浮細胞 PCV 值較高，但兩處理間仍無明顯差異，直到培養後第 28 天，由於 100 rpm 處理之細胞 PCV 值下降，致使兩處理結果開始呈現差異。在培養 28 天後利用 80 及 100 rpm 振盪速率處理的細胞 PCV 值分別約為 12.5 及 11.2 mL。

細胞接種密度試驗結果如圖 3 所示，當細胞接種密度為 6% (250-mL 錐形瓶) 懸浮細胞無明顯生長，經 4 週培養後細胞呈現褐化狀態。細胞接種密度為 12% (125-mL 錐形瓶) 經 4 週培養後之細胞乾重約為 0.26 g，而同樣 12% 細胞接種密度但培養容器為 250-mL 錐形瓶得到之細胞乾重為 0.51 g。細胞接種密度為 24% (125-mL 錐形瓶) 之懸浮細胞初期生長較為快速，在第 3 週後即可得最大乾重，但在培養 4 週後其細胞乾重與使用相同 125-mL 容器但接種密度為 12% 之處理並無顯著差異。

誘引劑對懸浮培養細胞生長增殖與柴胡皂苷含量之影響

誘引劑試驗結果如表 1 所示，就細胞鮮重而言，處理 1 週後各誘引劑處理之細胞生長與對照組相較均未受到明顯影響，僅 JA 與 ABA 高濃度處理相較於對照組呈現顯著降低 (P < 0.05) 的結果；在處理 2 週後，以無菌水對照組之細胞鮮重最高為 4.41 g/瓶，0.2% 乙醇對照組之細胞鮮重次之為 3.81 g/瓶，其餘各誘引劑處理之細胞鮮重介於 1.90–3.42 g/瓶，誘引劑間呈現顯著差異。若再就各誘引劑濃度效應而言，在培養 2 週後僅 1000 mg/L 較 500 mg/L YE 處理在濃度增加時對細胞鮮重有明顯抑

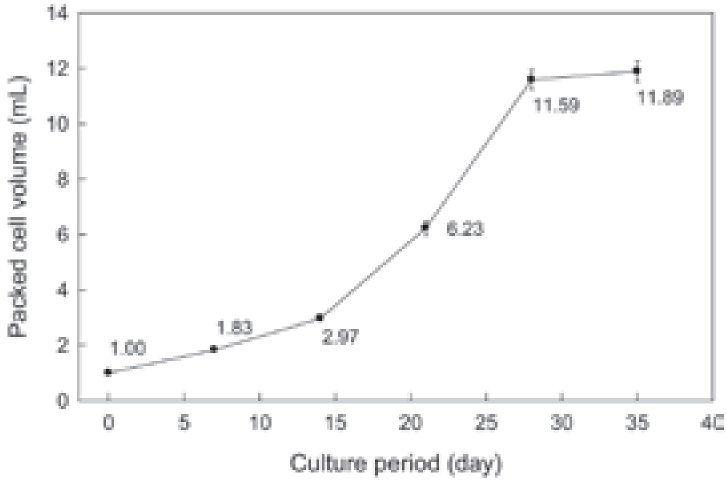

圖 1. 高氏柴胡懸浮細胞之生長增殖曲線。將 1 mL PCV 細胞添加於 24 mL 含有 0.2 mg/L 2,4-D 之 1/2 MS 液態培養基中以 100 rpm 振盪培養 5 週之生長情形，每週取樣調查 5 重複，圖中誤差線為標準誤差。

Fig. 1. Growth curve of cell suspension cultures of *Bupleurum kaoi*. One mL PCV cells were inoculated into a 24-mL 1/2 MS medium containing with 0.2 mg/L 2,4-D. Cultures were incubated at 25℃ on a shaker at 100 rpm and 5 flasks were sampled weekly for five weeks to measure the packed cell volume. Vertical bar indicates standard error of mean.

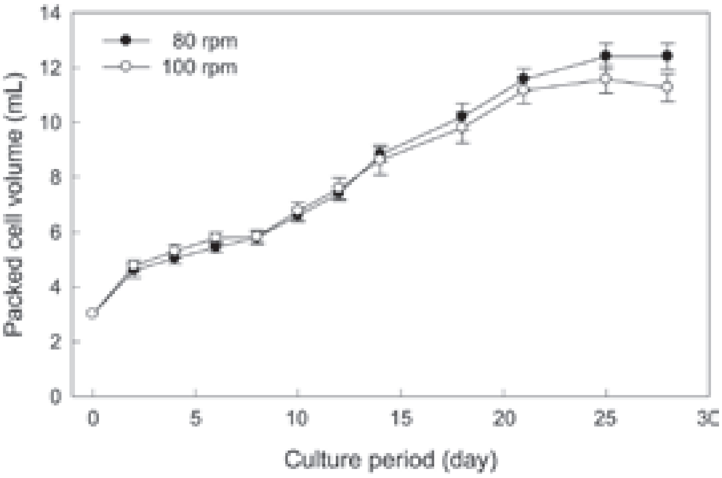

圖 2. 高氏柴胡懸浮細胞於不同振盪速度中生長之情形。接種 3 mL PCV 細胞於 22 mL D2 液態培養基之 125-mL 有臂錐形瓶，每 2 天調查細胞 PCV 值，共培養 4 週之細胞生長情形，每處理調查 3 重複，圖中誤差線為標準誤差。

Fig. 2. Effect of speed of shakers (80 and 100 rpm) on cell proliferation of *Bupleurum kaoi*. Cells grew in a 1/2 MS basal medium containing 0.2 mg/L 2,4-D. Cultures were sampled at 2-day-interval for 4 weeks, 3 flasks/sampling date/treatment, and measured the packed cell volume. Vertical bar indicates standard error of mean.

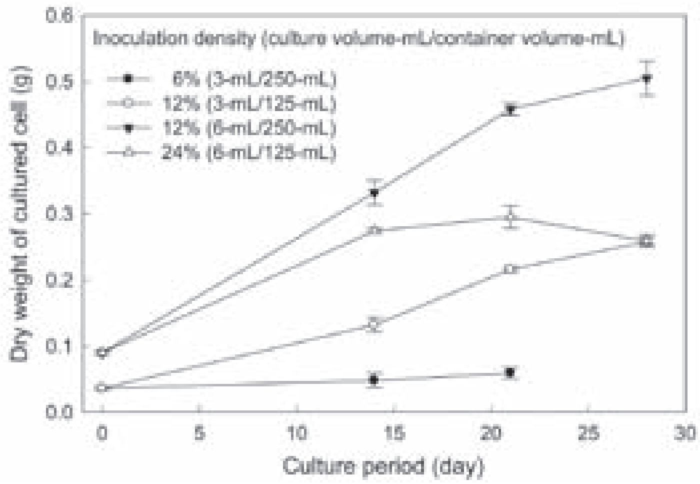

圖 3. 不同細胞接種密度下高氏柴胡懸浮細胞生長之情形。以 3 mL 及 6 mL PCV 接種於 125-mL 或 250-mL 之錐形瓶，兩種容器內之培養總體積分別為 25 mL 及 50 mL。懸浮細胞在培養後第 2、3 及 4 週後調查乾重，每處理調查 3 重複，圖中誤差線為標準誤差。

Fig. 3. Effect of inoculum density on cell proliferation of *Bupleurum kaoi*. Cells grew in a 1/2 MS basal medium containing 0.2 mg/L 2,4-D. Packed cell volume (PCV) of 3 mL or 6 mL were added into the culture medium to make a total 25 mL culture volume in the 125-mL flask for inoculum density of 12% and 24%, respectively. Larger culture medium volume of 50 mL in the 250-mL flask was also conducted by adding 3 mL or 6 mL PCV cultures to make 6% and 12% inoculum density, respectively. Data of cell dry weight was collected on 2nd, 3rd, and 4th week, 3 flasks/sampling date/treatment. Vertical bar indicates standard error of mean.

表 1. 誘引劑酵母抽出物、茉莉酸與離層酸在添加 1 及 2 週後對高氏柴胡懸浮細胞生長之影響
Table 1. Effect of elicitors on cell proliferation of *Bupleurum kaoi* after 1 and 2 week(s) coculture [z]

Elicitor (Con.)	Fresh weight (g/flask)		Dry weight (g/flask)	
	1 wk	2 wks	1 wk	2 wks
Control-1 (H_2O)	3.74 ± 0.28 a [y]	4.41 ± 0.20 a	0.40 ± 0.013 a	0.38 ± 0.008 a
Control-2 (0.2% EtOH)	3.29 ± 0.33 a	3.81 ± 0.11 b	0.37 ± 0.024 b	0.36 ± 0.003 b
YE (500 mg/L)	3.49 ± 0.05 a	3.42 ± 0.10 c	0.39 ± 0.004 ab	0.38 ± 0.009 a
YE (1000 mg/L)	3.33 ± 0.36 ab	3.05 ± 0.10 d	0.36 ± 0.016 bc	0.36 ± 0.010 b
JA (0.119 mM)	3.16 ± 0.30 ab	2.07 ± 0.00 f	0.32 ± 0.006 d	0.26 ± 0.006 d
JA (1.19 mM)	3.10 ± 0.40 b	1.90 ± 0.10 f	0.28 ± 0.016 e	0.22 ± 0.002 e
ABA (5 μM)	3.11 ± 0.45 ab	2.86 ± 0.16 e	0.36 ± 0.010 bc	0.36 ± 0.009 b
ABA (50 μM)	2.95 ± 0.28 b	2.81 ± 0.13 e	0.33 ± 0.022 cd	0.34 ± 0.006 c

[z] Cultures of *Bupleurum kaoi* were grown in a 1/2 MS basal medium containing 0.2 mg/L 2,4-D for 3 weeks on a shaker at 80 rpm and then treated with various concentration of YE, JA and ABA for 1 or 2 week(s). Autoclaved water (Control-1) and 0.2% EtOH (Control-2) in the medium were used as controls.

[y] Values are mean ± standard error and means within a column followed by the same letter(s) are not significantly different at 5% level by LSD test. There were 3 replicate flasks per treatment.

制，提高 JA 與 ABA 濃度對鮮重影響並不顯著。在細胞乾重方面仍以無菌水對照組之細胞乾重較高，誘引劑中以 YE 處理有較高之細胞乾重，ABA 處理次之，而以 JA 處理之細胞乾重最低。

在柴胡皀苷含量方面，表 2 顯示當懸浮細胞培養至第 3 週時之 SSa 與 SSd 加總含量為 0.076 mg/g dry wt (DW)，此時分別進行誘引劑(處理組)、0.2% 乙醇 (對照組) 與無菌水 (對照組) 之添加；當懸浮細胞培養至第 4 週時，無菌水對照組之 SSa 與 SSd 之加總含量為 0.098 mg/g DW，而 0.2% 乙醇對照組為 0.092 mg/g DW，三種誘引劑處理組之 SSa 與 SSd 之加總含量均高於對照組，500 mg/L YE、0.119 mM JA 與 5 μM ABA 處理組柴胡皀苷依序約為 0.18、0.19 與 0.20 mg/g DW，分別為無菌水對照組之 1.84、1.94 與 2.04 倍。依柴胡皀苷產量而言，則 500 mg/L YE 與 5 μM ABA 處理效果相當，分別為 3.51 及 3.58 mg/L，約為對照組之 1.79 與 1.83 倍，0.119 mM JA 處理效果次之為 3.04 mg/L，約為對照組之 1.55 倍。

酵母抽出物對懸浮培養細胞生長增殖之影響

以 500 mg/L YE 處理懸浮細胞 1 週及 2 週後對柴胡皀苷誘導累積結果顯示 (圖 4)，添加 500 mg/L YE 對於懸浮培養細胞之柴胡皀苷累積確有促進效果，施用 1 週後即可測得細胞之 SSa 與 SSd 加總含量約 0.45 mg/g DW，相較於對照組 SSa 與 SSd 加總含量 (0.21 mg/g DW)，增加約 2.14 倍；而 YE 施用達 2 週後其細胞之 SSa 與 SSd 加總含量達 1.67 mg/g DW，約為對照組細胞之 SSa 與 SSd 加總含量 (0.6 mg/g DW) 的 2.78 倍。

討　論

建立不同物種懸浮細胞培養之生長曲線除有助於了解各別物種細胞之生長習性外，更可利用細胞特有之生長週期決定細胞培養操作策略，例如繼代培養之最佳時期、添加誘引劑時機以及瞭解細胞內二次代謝物累積的情形作為收穫時間的參考等 (Chen & Chen 2000; Chueh *et al*. 2000)。本研究結果顯示，高氏柴胡懸浮細胞培養於 D2 液態培養基以 100 rpm 振盪速度於低光照環境中培養，其細胞增殖變化呈 S

表 2. 誘引劑酵母抽出物、茉莉酸與離層酸施用 1 週後對高氏柴胡懸浮細胞內柴胡皂苷含量之影響

Table 2. Content of saikosaponins in cell suspension cultures of *Bupleurum kaoi* after treating with elicitors for 1 week

Treatment [z]	Saikosaponins content (mg/g) SSa [y]	SSd	SSa + SSd (A)	Cell production (B) (g/L)	Productivity (A) × (B) (mg/L)
Control	0.050 ± 0.002 d [x]	0.026 ± 0.006 c	0.076	17.0	1.292
Control-1 (H_2O)	0.066 ± 0.006 c	0.032 ± 0.006 c	0.098	20.0	1.960
Control-2 (0.2% EtOH)	0.062 ± 0.003 c	0.030 ± 0.004 c	0.092	19.0	1.748
500 mg/L YE	0.142 ± 0.004 a	0.038 ± 0.003 b	0.180	19.5	3.510
0.119 mM JA	0.134 ± 0.003 a	0.054 ± 0.006 b	0.190	16.0	3.040
5 μM ABA	0.109 ± 0.004 b	0.090 ± 0.006 a	0.199	18.0	3.582

[z] Cultures of *Bupleurum kaoi* were grown in a 1/2 MS basal medium containing 0.2 mg/L 2,4-D, on a shaker (80 rpm) for 3 weeks and then treated with elicitors (500 mg/L YE, 0.119 mM JA or 5 μM ABA) for 1 week. Autoclaved water (Control-1) and 0.2% EtOH (Control-2) in the medium were used as controls.

[y] SSa: saikosaponin a; SSd: saikosaponin d.

[x] Values are mean ± standard error and the means within a column followed by same letter(s) are not significantly different at 5% level by LSD test. There were 3 replicate flasks per treatment.

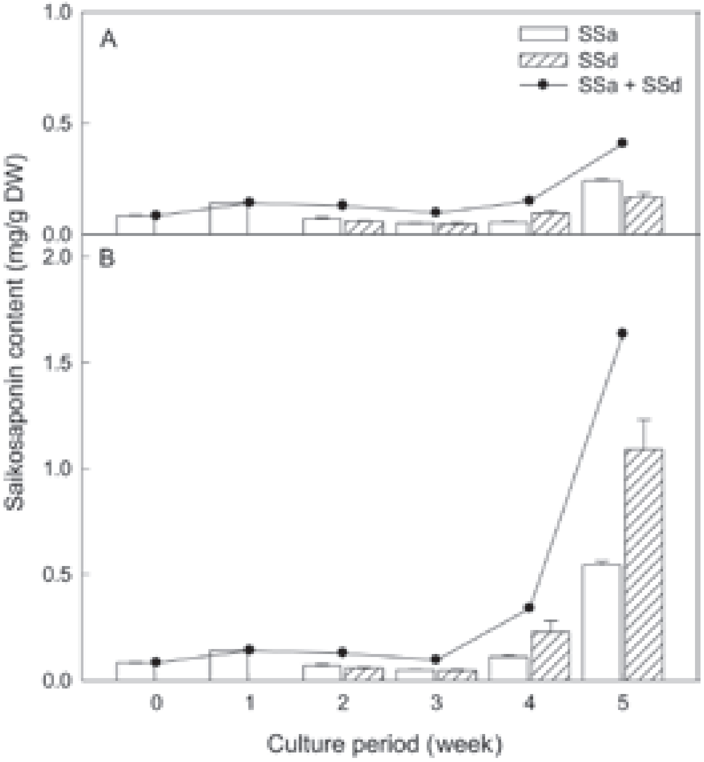

圖 4. 高氏柴胡懸浮細胞培養於添加 0.2 mg/L 2,4-D 之 1/2 MS 基本培養基中 3 週後施用無菌水 (A) 與 500 mg/L YE (B)，繼續培養 1 及 2 週後細胞之柴胡皂苷單位含量之變化，每處理調查 3 重複，圖中誤差線為標準誤差。

Fig.4. Changes of saikosaponins content of *Bupleurum kaoi* in cultures grown in a 1/2 MS basal medium containing 0.2 mg/L 2,4-D for 3 weeks and then treated with autoclaved water (A) and 500 mg/L YE (B) for 1 or 2 weeks. There were 3 replicate flasks per treatment. SSa: saikosaponin a; SSd: saikosaponin d.

型生長曲線 (圖 1)，其中 0–2 週為緩慢生長期 (lag phase)、2–4 週為快速生長期 (log phase) 及 4 週以後之生長靜止期 (stationary phase)，而後細胞即開始呈現褐化現象，因此判斷其繼代培養時間應在培養 3–4 週後較適宜。

振盪速率主要影響液態培養基中氣體的交換，若振盪轉速過慢可能導致培養細胞因氣體交換不足而死亡，振盪速率太快則容易造成細胞間彼此的撞擊而影響生長；此外，懸浮細胞之生長速率也會受到細胞接種密度的影響，接種密度過低，易因缺乏細胞相互間的支持造成細胞生長過於緩慢或死亡；若接種密度太高，則可能因為營養的迅速耗盡，以及空間的不足而無法縮短生長期，達到最高細胞產量，所以尋求適當振盪速率與細胞接種密度對提高懸浮培養的效率十分重要 (Chang *et al*. 1993; Chueh *et al*. 2000)。台灣龍膽 (*Gentiana davidii*) 懸浮細胞於80–100 rpm轉速培養時，細胞生長情形最佳，轉速過快 (120 rpm) 或過慢 (60 rpm)，對細胞生長均產生不利影響 (Chueh *et al*. 2000)。Chang *et al*. (1993) 報告指出台灣白芷 (*Angelica dahurica*) 懸浮細胞適合培養於 120 rpm 之振盪速率，其生長速率較以 100 及 80 rpm 培養之細胞為高。本試驗結果顯示高氏柴胡 (*B. kaoi*) 懸浮細胞培養達第 4 週後，80 rpm 振盪處理所得之細胞 PCV 值較 100 rpm 處理為高 (圖 2)，顯示不同物種細胞應有其適合的振盪培養速度。Chang *et al*. (2004) 於台灣紅豆杉 (*Taxus mairei*) 細胞培養研究指出，在 2–5% 低密度 (25–50 g/L) 接種條件下，細胞體積增加效果較佳。Chang *et al*. (1993) 於台灣白芷 (*A. dahurica*) 懸浮細胞培養研究指出，20% (v/v) 細胞接種密度具有最佳增殖效果，較低接種密度培養 (2–4% v/v) 則會延長其生長週期。本研究結果顯示高氏柴胡以12%細胞接種密度 (250-mL 錐形瓶)，具有較佳增殖效果，較高 (24%) 或較低 (6%) 的細胞接種密度均不利於高氏柴胡 (*B. kaoi*) 細胞之生長增殖；另外在相同細胞接種密度 (12%) 條件，以不同大小容器培養之細胞增殖效率也不相同，顯示不僅細胞接種密度會影響細胞培養效率，培養容器的容積大小也是影響細胞增殖的因子之一 (圖 3)。

許多研究報告指出，植物細胞培養過程中添加誘引劑可誘使其二次代謝物產量增加 (Radman *et al*. 2003; Mulabagal *et al*. 2004)。近年來，人參皂苷 (Lu *et al*. 2000)、紫杉醇 (Chang *et al*. 2004)、青蒿素 (artemisinin) (Baldi & Dixit 2008) 與丹參酮 (Chen & Chen 2000) 等重要藥用成分均有利用誘引劑促進培養細胞之藥用成分生產的成功實例。本研究結果顯示，施用 YE、JA 或 ABA 均會促進高氏柴胡懸浮細胞中柴胡皂苷的累積，然而在較高濃度或較長時間處理下，則顯示有抑制細胞生長的結果 (表 1)。誘引劑種類對二次代謝物累積具有不同的效果，添加 YE 可提高丹參 (*Salvia miltiorrhiza*) 細胞之丹參酮含量 (Chen & Chen 2000)。人參皂苷 (Lu *et al*. 2000) 與紫杉醇 (Wang *et al*. 2001) 可因為添加 YE 而大量形成。添加茉莉酸 (JA) 在人參 (*P. ginseng*) 細胞培養中，人參皂苷含量可隨著 JA 濃度的增加而增加 (Lu *et al*. 2000)。Baldi & Dixit (2008) 指出添加甲基茉莉酸 (Methyl Jasmonate, MJ) 於青蒿 (*Artemisia annua*) 細胞培養，對於青蒿素 (artemisinin) 的誘導效果最佳。金英花 (*Eschscholtzia californica*) 之細胞培養則顯示，MJ 對於金英花細胞中二氫血根鹼 (dihydrosanguinarine) 的誘導效果較佳，但血根鹼 (sanguinarine) 累積則以添加 YE 處理最好 (Cho *et al*. 2008)。Li *et al*. (2003) 於丹參細胞培養報告指出，添加 YE 可能導致 ABA 含量增加而使細胞生長受抑制，但卻能提高丹參酮含量。本研究結果亦顯示，直接施用 ABA 對促進高氏柴胡懸浮培養細胞中柴胡皂苷 SSa 與 SSd 的累積效果最

佳，雖然添加 YE 對提高柴胡皂苷的效果稍低於 ABA，但 YE 處理對細胞生長之抑制影響較小，因此在整體柴胡皂苷產量上與 ABA 相近，呼應 Li *et al*. (2003) 之試驗結果 (表 2)。

細胞培養中加入誘引劑雖可增加其二次代謝物產量，但誘引劑亦有可能抑制懸浮細胞的增殖 (Radman *et al*. 2003)。Chen & Chen (2000) 與 Li *et al*. (2003) 研究指出，不當濃度之 YE 與 JA 處理對丹參細胞生長具有相當大的抑制效果，因此對細胞二次代謝的生產而言，誘引劑的最適添加時機是在細胞生長週期中的快速生長後期 (late log phase) 或生長靜止前期 (early stationary phase)，可減少對細胞生長的影響；此外添加誘引劑的劑量與處理時間對於二次代謝物形成也極為重要 (Lu *et al*. 2000; Wang *et al*. 2001)。Chen & Chen (2000) 研究指出，生長快速之丹參細胞系僅含有少量丹參酮，但是當此細胞系培養於含 YE 的培養基後則可大量形成丹參酮，於是建議以兩階段培養 (two-stage cultural process) 方式進行丹參酮大量生產。然而 Lu *et al*. (2001) 研究指出，0–3 g/L YE 於細胞培養之第 1 天即加入，對人參細胞生長並無顯著影響，但在細胞培養達 5、10 及 15 天後再加入 YE，其細胞生長情形均較接種時即加入 YE 之處理為差，MJ 處理也有相似的趨勢。本研究結果顯示 YE、JA 及 ABA 此三種誘引劑處理均會抑制高氏柴胡懸浮細胞生長增殖，其中以 YE 處理對細胞生長的影響較輕微，而 JA 及 ABA 處理對高氏柴胡細胞生長有較大抑制作用 (表 1)；若以柴胡皂苷 SSa 與 SSd 之加總含量來看，顯示三種誘引劑之處理效果相當，且均高於對照組；但以成分產量而言，則 YE 與 ABA 處理效果相當，均較 JA 處理為佳，三種誘引劑處理組之柴胡皂苷產量均高於對照組，顯示三種誘引劑雖然對細胞之生長有影響，但均能促進及提高柴胡皂苷之累積 (表 2)。

本研究後續採用對於細胞生長抑制較輕微的 YE 進行進一步試驗，結果顯示在細胞培養 3 週後施用 500 mg/L YE 對細胞生長並無明顯抑制作用 (資料未列)，但具有促進柴胡皂苷累積之效果，在施用 1 週及 2 週後其細胞之柴胡皂苷含量分別為對照組之 2.14 及 2.78 倍；在圖 4B 中可以看到 YE 處理後 1 週細胞之柴胡皂苷尚未大量累積，處理 2 週後柴胡皂苷大幅上升。上述結果顯示，高氏柴胡細胞懸浮培養可藉由利用細胞生長之中後期施用 YE 誘引劑，並繼續培養 2 週後，提高柴胡皂苷之生產。表 2 結果顯示 500 mg/L YE 處理 1 週後柴胡皂苷含量為 0.18 mg/g，而圖 4 結果顯示在同樣處理時間下之柴胡皂苷含量可達 0.45 mg/g；根據本研究之接種試驗方法與結果顯示，有可能導因於不同容積大小之培養容器，但此推論仍需進一步研究證實。此外，由於 500 mg/L YE 處理 2 週後之懸浮細胞已呈現褐化現象，因此未能持續觀察後續柴胡皂苷累積結果 (圖 4)。

藥用植物建立細胞懸浮培養系統已有眾多例子，然實際應用於商業化生產者卻寥寥可屬，其中有效成分之經濟價值固然是影響能否商業化的主要原因之一，然而植物細胞的低增殖率以及有效成分過低則是需要克服的技術問題。許多研究報告指出，藉由分化器官培養 (differentiated organ culture)、細胞固定化培養 (immobilized cell culture) 與應用生物反應器 (bioreactor) 生產技術，均是達成增產目標的有效方法 (Gao *et al*. 2001; Tripathi & Tripathi 2003)。本研究結果顯示，雖然藉由目前懸浮細胞培養系統所得之柴胡皂苷含量現階段仍屬偏低，但未來可經由篩選高產細胞系及優化培養條件，以及誘引劑選擇、處理時機與時間等技術及配合培養操作策略，加上利用生物反應器大量培養生產之優點，應有助於利用細胞培養生產柴胡皂苷系統之實用性，此一模式的建立亦可作為其他重要藥用植物相關研究之參考依據。

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Influence of Elicitors on Cell Proliferation and Saikosaponin Production of *Bupleurum kaoi* in Suspension Culture[1]

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Abstract

Chen, U. C., C. N. Hsia, M. S. Yeh, C. Y. Tsao, and H. S. Tsay. 2012. Influence of elicitors on cell proliferation and saikosaponin production of *Bupleurum kaoi* in suspension culture. J. Taiwan Agric. Res. 61:112−123.

Bupleurum kaoi, a native medicinal plant in Taiwan which contains saikosaponins higher than other commercial *Bupleurum* species, was chosen for producing saikosaponins *in vitro*. Cell suspension cultures of *B. kaoi* were cultured in a half-strength MS liquid medium containing 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and used for studying effect of speed of shaker, size of flasks and inoculum density on cell proliferation as well as studying effect of elicitors, yeast extract (YE), abscisic acid (ABA), and jasmonic acid (JA), on saikosaponin production. Results showed that cell proliferation of *B. kaoi* in suspension cultures was higher using shaker speed at 80 rpm than that of 100 rpm with peak period of cell growth at 3−4 weeks. Cell proliferation of *B. kaoi* was also affected by inoculum density and flask size, with the optimum inoculum density of 12% by adding 6 mL packed cell volume (PCV) cultures in 250-mL flasks. Three elicitors (YE, ABA, and JA) tested in 3-week-old suspension cultures of *B. kaoi* were found reducing cell fresh weight and dry weight significantly. However, one week after the treatments of elicitors, saikosaponin contents and its productivity increased 1.9−2.0 folds and 1.6−1.8 folds, respectively. Further study using 500 mg/L YE as elicitor in cultures of *B. kaoi* for two weeks, similar increasing tendency on saikosaponin contents and its productivity was found with 2.1-fold and 2.8-fold, respectively. This study concludes that adding 500 mg/L YE into cell suspension cultures of *B. kaoi* after the lag phase of cell cycle for one to two weeks would not reduce cell growth but increase saikosaponin production.

Key Words: *Bupleurum kaoi*, Medicinal plant, Cell suspension culture, Saikosaponin, Elicitor.

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台灣農業研究 (J. Taiwan Agric. Res.) 61(2):124–131 (2012)

蝴蝶蘭貯運前藥劑處理對黃葉病發生之影響 [1]

廖國均 [2] 謝廷芳 [3,4] 陳宏榮 [3]

摘　　要

廖國均、謝廷芳、陳宏榮。2012。蝴蝶蘭貯運前藥劑處理對黃葉病發生之影響。台灣農業研究 61:124–131。

由 *Fusarium solani* 所引起的蝴蝶蘭黃葉病為台灣蝴蝶蘭海運外銷貯運耗損的主要原因之一。本研究以大白花 (*Phalaenopsis* Sogo Yukidian 'V3') 做為供試材料，測試於模擬貯運前處理撲克拉錳與幾丁聚糖對黃葉病發生之影響。試驗分為製造傷口及無傷口二組接種黃葉病菌，接種後以紙箱包裝，並靜置於 18℃ 黑暗冷藏庫中 20 與 26 天，觀察病害發生情形。試驗結果顯示，第一次試驗模擬貯運 20 天後，噴施撲克拉錳 (prochlorate manganese) 與幾丁聚糖 (chitosan) 後再行傷口接種病原菌之處理均可有效降低黃葉病的罹病度 (disease severity)，模擬貯運 26 天後撲克拉錳仍具抑病效果；而未行傷口接種組之處理間罹病度無差異。第二次及第三次試驗結果顯示，噴施撲克拉錳及幾丁聚糖後再接種病原菌之處理均無法有效降低模擬貯運後蝴蝶蘭黃葉病的罹病度。綜合二年三次試驗結果顯示，於貯運前只噴施一次撲克拉錳藥劑或幾丁聚糖並無法穩定且顯著地抑制蝴蝶蘭黃葉病的發生，有待進一步篩選其他更有效的藥劑或施用方法。

關鍵詞：蝴蝶蘭、模擬貯運、黃葉病、撲克拉錳、幾丁聚糖。

前　　言

蝴蝶蘭為台灣四大旗艦農產品之一，2010 年蝴蝶蘭的外銷產值為 6,668 萬 3 千美元，占農產品出口值的 1.68%，占花卉總出口值之 44.6% (Directorate General of Customs 2011, http://www.customs.gov.tw/StatisticWeb/IESearch.aspx)。自 2004 年 6 月 14 日美國正式同意台灣蝴蝶蘭帶介質輸美後，蘭苗銷美數量大幅增加，由 2004 年的 26,377 kg、價值 22,740 千元增加至 2010 年的 563,670 kg、價值 173,303 千元，2010 年更達我國蝴蝶蘭出口值的 41.6% (Directorate General of Customs 2011)。儘管成績亮眼，不過在空運價格昂貴之下，業者多採行海運方式出貨，農業試驗所研發的海運貯運技術普獲業者採用，可提昇到貨品質，並降低耗損率至 5% 以下 (Huang &

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Huang 2007)。目前蝴蝶蘭外銷海運耗損的主要原因不外乎有黃葉病、軟腐病、葉片黃化、葉片脫落等 (Huang & Huang 2007)，這些現象不僅影響賣相，嚴重者更必須丟棄，無疑增加業者的損失。蝴蝶蘭黃葉病為貯運損耗的主因之一，係由鐮孢菌 *Fusarium solani* (Martius) Snyder & Hansen (有性世代 *Nectria haematococca* Berkeley & Broome) 所引起，是現今蝴蝶蘭栽培相當棘手的真菌性病害 (Hsieh *et al*. 2007; Su *et al*. 2010)。*F. solani* 屬兼性寄生真菌，大部分行腐生生活，待環境適宜時，侵入莖基部或葉鞘，侵入後至發病約需 1–2 個月，當發現黃葉病徵時，往往葉鞘已被感染多時，防治時機掌握不易 (Hsieh *et al*. 2007)。黃葉病的診斷可由下位葉呈現失水狀或已呈黃化狀，判斷其黃化情形常常是由葉基部向上黃化，與正常葉片老化及失水的狀況不同，此時檢視葉鞘基部，可見黑褐色至黑色腐敗狀圓斑或不規則斑，其上有時出現肉鮭色圓球形子囊殼 (*F. solani* 的有性世代構造)，即可判定為鐮孢菌引起之蝴蝶蘭黃葉病，此時的蝴蝶蘭經濟價值降低，植株會慢慢衰弱 (Hsieh *et al*. 2007)。

蝴蝶蘭黃葉病與栽培管理有很大的關係，一般蝴蝶蘭栽培的適溫環境下亦適合本病害之發生，欲調控溫度防治本病之發生並不容易。蝴蝶蘭黃葉病可於介質中存活數年之久，鐮孢菌以無性孢子、菌絲或休眠構造如厚膜孢子存活於介質及植物殘體中，可藉由介質、水分移動、灰塵、栽培器具、工具及鞋子等傳播，若一遇到適宜的環境和感病的寄主出現時，又可感染危害 (Hsieh *et al*. 2007)。防治本病害可由栽培介質消毒及環境衛生管理下手，栽培介質最好於種植前數天以蒸熱方式 (80℃、30 分鐘) 進行消毒，栽培前之環境衛生管理非常重要，地面、床架、水牆等應以消毒水進行消毒，並隨時丟棄罹病植株。當病害發生時仍須以藥劑進行防治，蝴蝶蘭黃葉病以 50% 撲克拉錳可濕性粉劑 6000 倍對鐮孢菌之抑制效果較佳 (Chen 2009)，其餘藥劑如 25% 撲克拉乳劑 2000 倍、25.9% 得克利乳劑 3000 倍及 60% 銅合腐絕可濕性粉劑 500 倍等皆可嘗試使用。此外，幾丁聚糖被認為可誘導植株產生系統性抗病 (Sun & Chuang 2010)，近年來亦為業者廣泛使用之防治資材。本研究之目的在於探討蝴蝶蘭成熟株在海運貯運前處理撲克拉錳與幾丁聚糖後對黃葉病發生之影響，以作為貯運前植株處理之參考依據。

材料與方法

試驗材料

本試驗供試材料為蘭科 (Orchidaceae) 蝴蝶蘭屬 (Phalaenopsis) 之大白花 (*Phalaenopsis* Sogo Yukidian ‘V3’) 蝴蝶蘭品種，種植於古坑鄉農業試驗所花卉研究中心具風扇水牆的玻璃溫室中。試驗時挑選植株生長勢、大小一致，外觀無病蟲害及外傷之健壯植株 240 棵，並集中放置於一處，避免受到其他病蟲害的侵擾及感染，影響實驗的準確性。

施用藥劑為 50% 撲克拉錳可濕性粉劑 (prochlorate manganese WP) 與 1% 幾丁聚糖溶液 (chitosan solution)，幾丁聚糖溶液之製備乃以 1 g 幾丁聚糖粉劑加入 100 mL 的 1% 醋酸溶液中混合而成。

接種用的蝴蝶蘭黃葉病菌 *F. solani* 係由農業試驗研究所植物病理組蔬菜花卉研究室蘇俊峰博士提供，接種前將培養於馬鈴薯瓊脂培養基 (potato dextrose agar, PDA, Difco™, Detroit, USA) 上 2–3 星期之孢子以無菌水洗下，並以 3 層經高壓滅菌的紗布過濾菌液，再以血球計數器調整菌量濃度，製備成 10^5 spore/mL 的孢子懸浮液備用。

試驗方法

本試驗分別於 2010 年 8 月份、12 月份及 2011 年的 7 月份進行 3 次重複試驗，每一次

試驗共分傷口及無傷口處理二組，每組各有噴施 50% 撲克拉錳粉劑 6000 倍、1% 幾丁聚糖 500 倍及水 (正對照) 三個處理，處理後再行接種病原菌，並以噴水不接菌者為負對照組，採完全逢機設計 (completely randomized design, CRD)，每處理 5 重複，每重複 6 盆蘭株，共計 240 盆 3.5 吋商業成熟大苗。

試驗前蝴蝶蘭栽培盆內的水草介質先行充分澆水，隔天分別以上述四種處理 (撲克拉錳、幾丁聚糖、水、水) 進行苗株葉面噴施，直至藥液或水全面佈滿全株。處理 3 日後，傷口處理組之植株於由下而上數的第二與第三位葉間之葉鞘上以 5 支昆蟲針成束人工針刺造成傷口 (4 面戳傷)，除水處理不接菌之對照組外，其餘立即以黃葉病菌孢子懸浮液噴施於傷口處進行莖基部接種；無傷口處理組，植株不針刺造成傷口，依上法進行接種，接種後隔日以自製的真空抽氣機由栽培盆底部將水草介質之水分抽離 (圖 1)，抽乾 3 分鐘後將植株置於溫室中，直至介質以濕度計 (HH2 Moisture Meter, Delta-T Devices Ltd., England) 測量之讀值降到 25% 左右，隨即開始裝箱，一箱裝 24 株，裝箱後移至 25℃ 冷房進行預冷處理，隔天移入 18℃ 冷藏庫進行模擬貯運，貯藏期間以 TR-72U 溫濕度記錄器 (Thermo Recorder, T & D Co., Japan) 紀錄貯藏期間溫濕度變化，分別於貯藏 20 天及 26 天時調查所有植株罹黃葉病與軟腐病的病株數，以及黃葉病發病級數，調查後丟棄罹患軟腐病之植株，不列入黃葉病發病度計算。

病害調查與統計分析

病害發病級數之紀錄分為 5 級：0 為無出現病徵；1 為傷口接種處出現黑化；2 為病徵大小由傷口處往外擴散 < 3 mm；3 為病徵大小由傷口處往外擴散 > 3 mm；4 為葉片出現黃化病徵 (Su *et al*. 2010)。罹病度 (%) = [(發病級

圖 1. 利用真空抽氣機抽離蝴蝶蘭栽培盆內水草介質之水分。

Fig. 1. Removing moisture from the growth substrate (sphagnum moss) of Phalaenopsis plants in pots by a vacuum machine.

數 × 該級數罹病株數)/總調查株數 × 4] × 100。

各處理所得罹病度數據之差異顯著性測驗，係利用 SAS 9.1 統計分析軟體進行變方分析 (analysis of variance, ANOVA) 及最小顯著差異性 (least significant difference, LSD) 測驗。

結　果

模擬貯運 20 天後蝴蝶蘭黃葉病發生情形

三次試驗的供試植株皆以人工接種病原菌方式測試撲克拉錳與幾丁聚糖對蝴蝶蘭黃葉病發生之影響，於 18℃ 之冷藏庫中靜置 20 天，以模擬貯運到美西海關時的狀況。貯運期間，根據溫濕度紀錄器的紀錄，貯藏環境的溫度在 18 ± 1℃，而相對濕度在 80 ± 5% 內，開箱後調查箱內蝴蝶蘭的發病級數與受軟腐病影響的病株數 (調查後隨即丟棄，不列入發病度評比)，第一次試驗調查結果顯示，傷口接種組噴施撲克拉錳與幾丁聚糖之處理可有效降低黃葉病之嚴重性，其發病度分別為 14.2% 與 23.3%，與水處理後接菌之對照組發病度為 40.8% 呈顯著性差異 ($P < 0.05$)；而無傷口接種組之各處理間黃葉病發病度並無顯著差異 (表 1)。比較有無傷口接種處理間之黃葉病發生情形發現，除以水噴施不接菌處理外，其餘處理間具有顯著差異，即傷口接種處理之發病度高於無傷口處理組。不接菌之對照組在有無傷口處理均出現黃葉病病徵，且與無傷口接種之各處理間呈不顯著差異，表示該病徵為原先植株帶菌所引起之背景值，據此可發現黃葉病菌在無傷口之情況下，不易造成病害 (表 1)。第二次及第三次試驗結果顯示，傷口接種組在處理撲克拉錳與幾丁聚糖後與接菌之對照組比較並未能顯著降低黃葉病的發病度；無傷口接種組之二個藥劑處理與接菌對照組間之黃葉病發病度無顯著差異，且發病度均低 (表 2、表 3)。另外，與第一次試驗相似，噴水不接菌處理之對照組，無論有無傷口，其黃葉病發病度並無顯著差異，且與無傷口接種組之其他處理間呈不顯著差異 (表 2、表 3)。

模擬貯運 20 天調查時，傷口處理者遭受軟腐病菌感染的植株最多，第一次試驗以傷口接菌者之對照組有 4 株發病，撲克拉錳處理組及不接菌對照組各有一株，無傷口接菌之對照組有一株發病；第二次試驗完全沒有出現軟腐

表 1. 藥劑處理對蝴蝶蘭模擬貯運後黃葉病發生之影響 - 試驗一 (2010 年 8 月)

Table 1. Effect of prochlorate manganese and chitosan on control of Fusarium yellow of Phalaenopsis caused by *Fusarium solani* (Test 1, August, 2010)

	Disease severity (%) [y]			
	20 d [x]		26 d	
Treatment [z]	Wound	Non-wound	Wound	Non-wound
Prochlorate manganese	14.2 b A [w]	3.3 a B	19.5 bc A	5.0 a B
Chitosan	23.3 b A	10.0 a B	30.8 ab A	11.7 a B
CK (inoculated)	40.8 a A	0.0 a B	44.0 a A	6.7 a B
CK (un-inoculated)	12.5 b A	8.3 a A	16.7 c A	11.8 a A

[z] Chemicals, 6000-fold dilution of 50% prochlorate manganese WP and 500-fold dilution of 1% chitosan, were used to treat the Phalaenopsis seedlings.

[y] Disease on each plant was rated, based on a scale of 0 to 4: “0” = no symptom, “1” = black necrosis at the inoculated site, “2” = black necrotic lesion < 3 mm, “3” = black necrotic lesion > 3 mm, “4” = leaf yellowing. Disease severity (DS) was calculated using the formula: DS (%) = [Σ (nd)/4T] × 100, where n = number of plants in each rating, d = disease rating scale (0 to 4), and T = total number of plants.

[x] Plants were incubated at 18℃ for 20 or 26 days.

[w] Means in each column followed by the same lower case letter or in each row followed by the same capital letter indicate no significant difference at 5% level by LSD test.

表 2. 藥劑處理對蝴蝶蘭模擬貯運後黃葉病發生之影響 - 試驗二 (2010 年 12 月)

Table 2. Effect of prochlorate manganese and chitosan on control of Fusarium yellow of Phalaenopsis caused by *Fusarium solani* (Test 2, December, 2010)

	Disease severity (%) [y]			
	20 d [x]		26 d	
Treatment [z]	Wound	Non-wound	Wound	Non-wound
Prochlorate manganese	29.2 a A [w]	0.8 a B	35.0 a A	3.3 a B
Chitosan	35.0 a A	2.5 a B	40.8 a A	8.3 a B
CK (inoculated)	36.7 a A	3.3 a B	40.9 a A	5.8 a B
CK (un-inoculated)	3.3 b A	0.0 a A	4.2 b A	3.3 a A

[z] Chemicals, 6000-fold dilution of 50% prochlorate manganese WP and 500-fold dilution of 1% chitosan, were used to treat the Phalaenopsis seedlings.
[y] Disease on each plant was rated, based on a scale of 0 to 4: "0" = no symptom, "1" = black necrosis at the inoculated site, "2" = black necrotic lesion < 3 mm, "3" = black necrotic lesion > 3 mm, "4" = leaf yellowing. Disease severity (DS) was calculated using the formula: DS (%) = [Σ (nd)/4T] × 100, where n = number of plants in each rating, d = disease rating scale (0 to 4), and T = total number of plants.
[x] Plants were incubated at 18℃ for 20 or 26 days.
[w] Means in each column followed by the same lower case letter or in each row followed by the same capital letter indicate no significant difference at 5% level by LSD test.

表 3. 藥劑處理對蝴蝶蘭模擬貯運後黃葉病發生之影響 - 試驗三 (2011 年 7 月)

Table 3. Effect of prochlorate manganese and chitosan on control of Fusarium yellow of Phalaenopsis caused by *Fusarium solani* (Test 3, July, 2011)

	Disease severity (%) [y]			
	20 d [x]		26 d	
Treatment [z]	Wound	Non-wound	Wound	Non-wound
Prochlorate manganese	26.8 a A [w]	0.8 a B	34.5 a A	4.2 a B
Chitosan	24.2 a A	0.0 a B	29.8 a A	3.3 a B
CK (inoculated)	27.0 a A	0.0 a B	34.8 a A	0.0 a B
CK (un-inoculated)	8.3 b A	0.0 a A	8.3 b A	3.3 a A

[z] Chemicals, 6000-fold dilution of 50% prochlorate manganese WP and 500-fold dilution of 1% chitosan, were used to treat the Phalaenopsis seedlings.
[y] Disease on each plant was rated, based on a scale of 0 to 4: "0" = no symptom, "1" = black necrosis at the inoculated site, "2" = black necrotic lesion < 3 mm, "3" = black necrotic lesion > 3 mm, "4" = leaf yellowing. Disease severity (DS) was calculated using the formula: DS (%) = [Σ (nd)/4T] × 100, where n = number of plants in each rating, d = disease rating scale (0 to 4), and T = total number of plants.
[x] Plants were incubated at 18℃ for 20 or 26 days.
[w] Means in each column followed by the same lower case letter or in each row followed by the same capital letter indicate no significant difference at 5% level by LSD test.

病株；第三次試驗時，傷口接菌之撲克拉錳處理組及接菌對照組各一株發病，而無傷口接菌的撲克拉錳處理組有一株發病，三次試驗以幾丁聚糖處理者皆未發生軟腐病。

模擬貯運 26 天後蝴蝶蘭黃葉病發生情形

模擬貯運 26 天後，將植株移置於催花房，隨即調查病害發生之情形，以模擬貯運至美東與荷蘭到貨時植株的狀況。貯運期間，根據溫濕度記錄器的紀錄，貯藏環境的溫度在 18 ± 1℃，而相對濕度在 75 ± 5 % 內，相較於模擬貯運 20 天之濕度略有些微降低的現象。開箱後調查蝴蝶蘭的黃葉病發病級數及遭受軟腐病為害的病株數。第一次試驗結果顯示傷口處理組噴施撲克拉錳可降低黃葉病發病度，與

接菌對照組呈顯著差異 ($P < 0.05$) (表 1)，然而第二次與第三次試驗結果顯示撲克拉錳與幾丁聚糖處理均無法有效降低病害之發生 (表 2、表 3)。另外，無傷口處理組之病害發生情形與貯運 20 天之結果相同，可見黃葉病菌在無傷口處理的情況下，不易引起病害。

討　　論

本研究第一次試驗顯示 50% 撲克拉錳可濕性粉劑對於黃葉病的防治效果顯著，幾丁聚糖次之，然而後續二次試驗二者之防治效果不彰，顯示以人工接種病原菌之方式在貯運前噴施一次藥劑並無法穩定降低病害的發生。另外，三次試驗發現在無傷口處理的情況下，於 26 天模擬貯運之發病度介於 0–11.8%，但均與有傷口不接菌之處理無顯著差異性，亦即本菌在無製造傷口的情況下不易侵入寄主植物。以黃葉病潛伏期長的特性研判 (Hsieh *et al*. 2007)，這些發病植株應該並非試驗接種所造成，而是接種前已帶菌，可作為試驗材料之背景值，並不影響數據之分析與判讀。由此可見，貯運前的栽培管理與病害防治成效影響出貨品質甚鉅。

本研究所使用的 50% 撲克拉錳可濕性粉劑為政府正式推薦用於防治由鐮胞菌所引起之作物病害的農用藥劑 (Fei *et al*. 2010)，撲克拉錳為固醇碳 14 去甲基作用抑制劑 (sterol C14-demethylation inhibiting fungicides) 中的咪唑類藥劑，與撲克拉同類，此類藥劑可與 microsomal cytochrome P-450 的特別型態結合，在不影響糖類及醋酸鹽氧化反應的狀況下，比嗎福 (morpholines) 更早抑制固醇生合成 (Liao 2005)。對於不同菌種，本藥劑可能有抑制孢子堆複製、減緩菌絲生長及抑制孢子發芽等作用 (Lee 2008)。一般而言，於田間進行防治藥劑之有效性評估時，採每隔 7 至 10 天噴施藥劑一次，連續三次再調查病害防治效果，本研究僅於貯運前處理藥劑一次，結果並未能有效降低貯運期間蝴蝶蘭黃葉病發生；另外，撲克拉錳並非系統性藥劑，本研究以人為製造傷口方式接種病原菌，病原菌容易藉由傷口侵入無藥劑保護的組織內。這些可能都是噴施藥劑無法穩定抑病的原因，未來應進行系統性藥劑之測試或篩選更有效的防治藥劑，並建立貯運前藥劑處理之技術。

幾丁聚糖或稱甲殼素 (chitosan) 乃是幾丁質 (chitin) 去乙醯基後所產生的衍生物，此藥劑並非殺菌劑，故無產生抗藥性菌株之虞，植物遭受病原菌、病毒及機械傷害時會誘導產生幾丁質酶 (chitinase) 對抗病原真菌的入侵 (Gupta *et al*. 1995; Chernin *et al*. 1997)，而施用幾丁聚醣可誘發植物產生抗病性反應 (Hirano *et al*. 1990; Vad *et al*. 1991)。其作用機制為病原菌侵入植物時，植物可分解真菌細胞壁中的 chitin，產生 chitosan，chitosan 可啟動植物的防禦系統，並且透過信號分子的傳遞產生干擾物質、酚類化合物等抗菌物質，抵抗病原菌的侵入 (Roby *et al*. 1987)。除此之外，幾丁質類物質對於其他微生物如細菌及真菌均具有抑菌效果 (Hirano & Nagao 1989)，以此對照試驗調查結果發現，幾丁聚糖處理組全未發生細菌性軟腐病應該有關。然而本研究結果顯示 chitosan 未達顯著的抑病效果，可能是 chitosan 處理植株誘導抗病性產生需要一段時間，2 到 3 天的時間仍不足以誘導啟動植物防禦系統。Sun & Chuang (2010) 以西方墨點法分析蝴蝶蘭組織中超氧化歧化酶 (superoxide dismutase，簡稱 SOD) 與病程相關蛋白 (pathogenesis-related protein 2，簡稱 PR2) 之含量，發現蝴蝶蘭處理 chitosan 與 harpin 均可誘導植體產生大量 SOD 蛋白，而經貯運後 SOD 蛋白仍可累積於所有處理之植株中，但經數週貯運過程使蘭苗處於生理逆境下，對照組植體亦累積大量的 SOD；以上述二種藥劑處理蘭苗，經貯運後發現

harpin 處理之蘭苗組織中 PR2 蛋白質累積量顯著增加，但 chitosan 處理組之蘭苗組織中 PR2 卻消失，貯運後 PR2 的累積量與罹病率呈反比，進一步進行接種試驗發現兩者皆可降低黃葉病罹病率，但 chitosan 產生的抗性會受貯運過程影響而降低抑病效果 (Sun & Chuang 2010)，此結果與本試驗測試 chitosan 的防病效果相仿。此外，*F. solani* 孢子於 500 倍 1% chitosan 溶液下其發芽管長度明顯長於 6000 倍撲克拉錳及無菌水處理 (未發表資料)，由此推斷，由於 chitosan 為一多醣物質，錯誤的濃度反而有利於真菌生長，過去也有一些未發表的誘導抗病實驗，因為使用高濃度的 chitosan，反而快速促進病害發生。未來可針對 chitosan 使用濃度與處理次數對黃葉病發生之影響進行深入探討。

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Effect of Chemical Treatment on Prevention of Fusarium Yellow of Phalaenopsis Seedlings under Simulated Conditions of Shipment[1]

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Abstract

Liao, G. J., T. F. Hsieh, and H. R. Chen. 2012. Effect of chemical treatment on prevention of Fusarium yellow of Phalaenopsis seedlings under simulated conditions of shipment. J. Taiwan Agric. Res. 61:124−131.

Fusarium yellow, caused by *Fusarium solani*, is a serious disease of *Phalaenopsis* spp. in shipment and it is one of the major factors affecting the export market of this flower crop in Taiwan. The objective of this study was to determine effect of chemical treatment on prevention of Fusarium yellow of Phalaenopsis seedlings under simulated conditions in shipment, using white-flower *Phalaenopsis* Sogo Yukidian 'V3'. Seedlings (18-month-old) were treated with 50% prochlorate manganese WP or 1% chitosan for 3 days and then inoculated with spore suspension (10^5 spore/mL) of *F. solani* with or without wounding of orchid plants. The wounding treatment was done by puncturing the leaf sheath of each orchid plant using a bundle of 5 needles. For the treatment of control, seedlings were treated with sterile water and inoculated with *F. solani*, with or without wounding. All the plants, treated or untreated control, were incubated in a growth chamber without lighting at 18℃ for 20 and 26 days and they were rated for disease severity, using a scale of 0 to 4 (0 = no symptom on leaf and 4 = leaf yellowing). Results of the first experiment showed that treatment of wounded plants with prochlorate manganese or chitosan for 20 or 26 days significantly ($P < 0.05$) reduced severity of Fusarium yellow of Phalaenopsis. However, the result of this experiment was not repeatable in the two subsequent experiments. The inconsistency of these experiments indicates that spraying Phalaenopsis plants with prochlorate manganese or chitosan once is inadequate for protection of plants from developing Fusarium yellow during the period of shipment for 20 or 26 days.

Key words: Phalaenopsis, Simulated shipment conditions, *Fusarium solani*, Prochlorate manganese, Chitosan.

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Complete Nucleotide Sequence of RNA2 of *Cucurbit chlorotic yellows virus* Isolated from Melons in Taiwan and Its Comparisons with Currently Existing Isolates[1]

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Abstract

Lin, Y. T, J. Y. Liao, C. H. Tsai, and T. C. Deng. 2012. Complete nucleotide sequence of RNA2 of *Cucurbit chlorotic yellows virus* isolated from melons in Taiwan and its comparisons with currently existing isolates. J. Taiwan Agric. Res. 61:132−143.

The nucleotides of genomic RNA2 of *Cucurbit chlorotic yellows virus* (CCYV) isolates CCYV-Erlun (JN126045) and CCYV-Yilan (JN126046) collected from Taiwan were completely sequenced. In addition to 5’- and 3’- terminal untranslated regions, a total of 8041 bp in length encoding genes of *p4.9* (132 nt), *HSP70h* (1671 nt), *p6* (165 nt), *p59* (1554 nt), *p9* (240 nt), *CP* (753 nt), *CPm* (1425 nt), and *p26* (642 nt) were determined by reverse transcription polymerase chain reaction (RT-PCR) and rapid amplification of 5' complementary DNA ends (5’-RACE). The CP gene-targeted primers for RT-PCR would be useful for improving the sensitivity and accuracy of CCYV detection. Comparative analyses of the nucleotide sequences of CCYV isolates with those of various geographic origins including Japan, Taiwan, China, and Sudan revealed that the identities are as high as 99% to 100% in respective genes of *HSP70h*, *CP*, *CPm* and entire RNA2. Entropy plotting demonstrated the variability of nucleotide sequences among two Taiwan isolates and an initial Japan isolate had only 12 divergent nt in a total of 8041 nt of RNA2, and the nucleotide sequence variance located mainly in the 5’- and 3’- terminal regions. The remarkable homogeneity and very close cluster of all CCYV nucleotide sequences available for the phylogenetic analyses indicate that all the currently existing CCYV isolates in the world were originated from the same source.

Key words: CCYV, RT-PCR, Phylogenetic analysis, Entropy plot.

Introduction

Cucurbit chlorotic yellows virus (CCYV) belongs to genus *Crinivirus* and family *Closteroviridae*, emerged in 2004 as a new disease in Japan (Gyoutoku *et al*. 2008). Usually, serious yellowing is the typical symptom of CCYV-infected plants and that causes damages to the yield and quality of cucurbit fruit production. CCYV can be transmitted by the B-type or Q-type tobacco whitefly (*Bemisia tabaci* L.) with semi-persistent manner (Gyoutoku 2008; Huang *et al*. 2010). Host range tested by inoculation with viruliferous *B. tabaci*, CCYV could infect

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plants of *Asteraceae*, *Chenopodiaceae*, *Convolvulaceae*, *Cucurbitaceace*, *Fabaceae*, and *Solanaceae* (Okuda *et al*. 2010). The name of CCYV was designated by Gyoutoku et al. (2009), but the nomenclature was formally set up with the evidence of entire CCYV genomic sequences and their organizations were determined by Okuda et al. (2010). The initial Japanese CCYV isolate consisted of two positive sense single-stranded RNAs, namely RNA1 and RNA2. RNA1 possesses four open reading frames (ORFs) encoding for papain-like protease, methyltransferase, RNA helicase 1 and RNA-dependent RNA polymerase (RdRp), respectively. RNA2 possesses eight ORFs encoding for hypothetical 4.9-kDa protein (p4.9), 70-kDa heat-shock protein (HSP70h), hypothetical 6-kDa protein (p6), hypothetical 59-kDa protein (p59), hypothetical 9-kDa protein (p9), coat protein (CP), minor coat protein (CPm) and hypothetical 26-kDa protein (p26) as shown at the top of Fig. 3 (Okuda *et al*. 2010).

CCYV was first found on melon (*Cucumis melo* L.) in Kumamoto Prefecture Kyushu, Japan in August 2004 (Gyoutoku *et al*. 2008), then it was also detected on cucumber (*Cucumis sativus* L.) grown in plastic houses in Saga Prefecture (Furuta *et al.* 2008). Till the winter of 2004, CCYV had spread to Miyazaki Prefecture and induced severe yellows disease of cucumber and melon (Kuno *et al*. 2008). From 2005 to 2007, CCYV was prevalent on cucurbit crops, especially cucumbers and melons, and the epidemics were widespread to the whole island of Kyushu in 2008 (Gyoutoku 2008). In September of 2008 the plague disseminated over 1000 km to the eastern Gunma in Kanto region and then spread to neighboring prefectures of Saitama, Tochigi, and Ibaraki in Honshu, Japan (Kuwabara *et al*. 2009; Okuda 2010). In this case, isolates of CCYV occurred in Gunma (Honshu) and those in Kumamoto (Kyushu) were considered to be derived from the same source (Kuwabara *et al*. 2009). CCYV could be distributed long distances within a short time due to the outsourced seedlings which carried CCYV-infected plants or viruliferous whitefly during the transportation. After invasive CCYV was established, transmission by *B. tabaci* accelerated the dissemination of the epidemics. When CCYV was emerged in Kyushu, the epidemics also scattered across the strait to Ehime Prefecture, Shikoku, and in 2009, spread to the Hiroshima and Kochi Prefectures (Okuda 2010).

Outside Japan, in October 2007, symptoms of CCYV infection were found on Hami melon (*C. melo* cv. Xuelihong) cultivated under tunnel in Nanhui, Shanghai, China and then the disease broke out around Shanghai area in 2009. The nucleotide sequence of heat shock protein 70-kDa homologue gene (*HSP70h*) of Shanghai origin CCYV (GenBank Accession No. HQ148667) had 100% identity with Japan isolate (Zeng *et al*. 2011). Gu *et al*. (2011) also reported that CCYV had occurred on plants of melon, watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] and cucumber grown in plastic houses in Shanghai area in 2008, and then spread over 13,000 ha and became prevalent in Shanghai, Ningbo (Zhejiang Province), and Shouguang (Shandong Province). The nucleotide sequence of cucumber isolate collected in Ningbo had 99.7% (*CP*), 99.9% (*CPm*) and 99.9% (*HSP70h*) nucleotide identities with the respective genes of Japanese isolate. First case of CCYV in Taiwan was discovered in 2009, in Lunbei County of Yunlin Prefecture (Tseng *et al.* 2009), then the epidemics have been found in melon, oriental pickling melon (*C. melo* L. var. *conomon*), cucumber, watermelon, pumpkin (*Cucurbita moschata* Duchesne), wax gourd (*Benincasa hispida* Thunb.), luffa (*Luffa cylindrica* Mill.), and bottle gourd [*Lagenaria siceraria* (Molina) Standl.] in all regions of Taiwan (Huang *et al*. 2010; Deng 2011). The RNA2 partial nucleotide sequence (GenBank Accession No. JF502222) of CCYV isolated from muskmelon in Taiwan shared 99% identity with Japanese isolate (GenBank Accession No. AB523789) (Li 2009). Recently, the presence of CCYV affecting melon and cucumber plants in Khartoum, Sudan was reported as the first case of CCYV outside Eastern Asia; however, the Sudanese CCYV nucleotide sequence had only negligible differences from Asian isolates (Hamed *et al*. 2011).

In this study, we have completed the sequencing of the full-length nucleotide of RNA2 of CCYV isolates collected from cucurbits in Taiwan. According to these data and comparing with those obtained from other currently existing isolates of CCYV in the world, phylogenetic relationships were analyzed and the genomic divergence was revealed by Entropy (Hx) plotting. In addition, a pair of primers targeting coat protein gene (*CP*) for RT-PCR shown to be useful for improving the CCYV detection is discussed.

Materials and Methods

Source of the virus

In the routine survey of cucurbit viral diseases, isolate of CCYV-Erlun was collected from muskmelon cultivated in open field of Erlun, Yunlin Prefecture (Lin *et al*. 2010) and CCYV-Yilan was collected from oriental type melon (*C. melo* L. var. *albidia* Makino) grown in the screen house of Jhuangwei, Yilan Prefecture (Deng *et al*. 2010). Leaves or fruits of plants with chlorosis and yellowing symptoms were sampled for indirect ELISA to confirm they were free from other viruses, including *Cucurbit aphid-borne yellows virus*, *Cucumber green mottle mosaic virus*, *Cucumber mosaic virus*, *Melon yellow spot virus*, *Papaya ringspot virus*, *Squash leaf curl virus*, *Watermelon silver mottle virus*, and *Zucchini yellow mosaic virus*. Moreover, an approximate 500-bp amplified fragment produced by RT-PCR using crinivirus-specific primer CCYV-F/R (Gyoutoku et al. 2009) was used to confirm the status of CCYV-infection in CCYV-Erlun and CCYV-Yilan. Both isolates were used as the source of CCYV for the entire nucleotide sequence of RNA2.

Design of specific primers for CCYV detection and full-length sequencing

With reference to the database of NCBI, U.S. National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/), the complete RNA2 nucleotide sequence of the initial Japan CCYV isolate (GenBank accession no. AB523789) (Okuda *et al*. 2010) was adopted to design specific primers for the RT-PCR amplification and full-length sequencing. By use of CLC Main Workbench v 5.71, a pair of primers CCYV-jF2 (5’-ACTGACAATAAACAAAACGATGAT-3’) and CCYV-jR2 (5’-TCTTTGCTCTCCATTATTTACTAC-3’) were designed to amplify a 733-bp fragment of the coat protein gene (*CP*). The other primers used in this study for RT-PCR were listed in Table 1.

Total RNA extraction and full genome amplification

Total RNA was extracted and purified from 100 mg of fresh leaf (CCYV-Erlun) or fruit (CCYV-Yilan) tissues using RNA extraction kit (Plant Total RNA Extraction Miniprep System, Viogene, CA, US) with the manufactures’ instructions.

Primer pairs listed in Table 1 were adopted to amplify respective gene regions, such as CCYV-F/RNA2-HSP-P59-R for a 2.4 kb region spanning *HSP70h*−*p59* gene, RNA2-P59-CP-F/RNA2-CP-R1 for a 2.45 kb region spanning *p59*−*CP* gene , CCYV-jF2/jR2 for a 733 bp region of *CP* gene, and CCYV-mcp-F/CCYV-RNA2-R for a 2.2 kb region spanning *CPm*−3’end. A mixture of 1 μL RNA, 4 μL primer (0.5 μM), 17 μL Diethylpyrocarbonate-treat-

Table 1. Nucleotide sequence and target site of primers used for RT-PCR and sequencing

Name	Sequence (5’−3’)	Target site [z]	Reference
RNA2-race0	GAGAAACCACGATCAATTCC	*Hsp70h*	Okuda *et al*. 2010
RNA2-race1	ACTCCGCCACATATAATGGT	*Hsp70h*	Okuda *et al*. 2010
RNA2-race2	CCAACCCATCTCTTCAAATC	*Hsp70h*	Okuda *et al*. 2010
RNA2-CP-R1	CTTTCAGAGCTTGGCACTTC	*CP*	Okuda *et al*. 2010
CCYV-F	AGTGCGTATGTCAATGG	*Hsp70h*	Gyoutoku *et al*. 2009
CCYV-R	GATAATCCTTCGCAGTG	*Hsp70h*	Gyoutoku *et al*. 2009
RNA2-HSP-P59-R	GATGCTGTGGGAATGTC	*Hsp70h*	This study
RNA2-P59-CP-F	GTGGAACCGAACCTGTTA	*p59*	This study
CCYV-jF	AGGCGGTACTACTTTATGCGTCTT	*CP*	This study
CCYV-jF2	ACTGACAATAAACAAAACGATGAT	*CP*	This study
CCYV-jR2	TCTTTGCTCTCCATTATTTACTAC	*CP*	This study
CCYV-mcp-F	TTCAAATAAAAATCGGTGTTCCTG	*CPm*	This study
CCYV-mcp-R	TATCGAAAAGCGCATGAGACTGTA	*CPm*	This study
CCYV-RNA2-R	GGCCTAGCTATGCTACTAACTA	3’-end of RNA2	This study

[z] *Hsp70h*: Heat shock protein 70-kDa homologue; *CP*: coat protein; *p59*: hypothetical 59-kDa protein; *CPm*: minor coat protein.

ed distilled water (DEPC-DDW), and AccuPower® RT-PCR PreMix (Bioneer, Daejeon, Korea), was placed into MultiGene Thermo Cycler TC-9600 (Labnet, Edison, NJ, USA) for amplification. The reactions were conducted at 70℃ for 5 min, 10℃ for 10 min, and 94℃ for 2 min, then repeated with 35 cycles of 94℃ for 60 s, 50℃ or 54℃ for 60 s, 70℃ for 60 s or 150 s before the final run at 70℃ for 20 min.

As nucleotide sequence of 5'-end, 5'-RACE system (5′ Rapid Amplification of cDNA Ends; Invitrogen, Carlsbad, CA, USA) was adopted to amplify the unknown fragments (Frohman *et al*. 1988).

Full length sequencing of RNA2

The small amount of gel DNA recovery kit (Gel Advanced Extraction Miniprep System; Viogene, CA, US) was used to recover and purify RT-PCR products which were then cloned to yT&A vector and ECOS101 competent cells (Yeastern Biotech, Taiwan). The plasmid DNA of transformed clones were extracted and analyzed for sizes of embedded fragments to confirm the expected nucleotides that were cloned before the nucleotides of DNA fragments embedded in these recombinant plasmids were sequenced by the Mission Technology Co., Taiwan. All the sequenced data of each fragment were integrated into a coherent RNA2 full-length nucleotide sequence.

Analysis of phylogenetic relationships

By means of alignment in ClustalX2 (http://www.clustal.org/), genomic nucleotide sequences of heat shock protein 70-kDa homologue (*HSP70h*), coat protein (*CP*), minor coat protein (*CPm*), and full-length or partial RNA2 of CCYV-Erlun and CCYV-Yilan were ccompared with other isolates of CCYV listed in Table 2. The other related criniviruses, such as *Lettuce chlorosis virus* (LCV) (Salem *et al*. 2009) and *Cucurbit yellow stunting disorder virus* (CYSDV) (Aguilar *et al*. 2003) were included for comparative analyses and *Beet yellows virus* (BYV) (Agranovsky *et al*. 1994) was set as an out-group control. Phylogenetic dendrograms were generated by neighborhood-joining method (MEGA4: Molecular Evolutionary Genetics Analysis, http://www.megasoftware.net/) and checked with Bootstrap by 1000 replicates. The variability of nucleotide sequence of CCYV RNA2 between a Japan isolate (AB523789) and two Taiwan isolates (JN126045, JN126046) were searched by BioEdit which resulted in generating Entropy (Hx) plots (Hall 1999).

Table 2. List of all CCYV RNA2-associated nucleotides available in the database of NCBI GenBank

Accession	Gene/Length	Host	Location/Isolate	Date	Reference
AB523789	Complete RNA2/8041 bp	Melon	Japan: Kumamoto/Initial isolate	2004	Okuda *et al*. 2010
JF502222	Partial RNA2/6632 bp	Muskmelon	Taiwan: Yunlin/CCYV-TW	Jun-2009	Li 2010
JN126046	Complete RNA2/8041 bp	Oriential melon	Taiwan: Yilan/CCYV-Yilan	Aug-2010	Deng *et al*. in press
JN126045	Complete RNA2/8041 bp	Muskmelon	Taiwan: Erlun /CCYV-Erlun	Feb-2011	Lin *et al*. 2011
JF807055	*HSP70h*/295 bp	Melon	Sudan/CCYV-3P	May-2009	Hamed *et al*. 2011
HQ148667	*HSP70h*/462 bp	Melon	China/CCYV-shjd	Oct-2009	Zeng *et al*. 2011
GU721111	*Hsp70h*/462 bp	*B. tabaci*	China: Shouguang/CCYV-btc	Nov-2009	Gu *et al*. 2011
GU721109	*Hsp70h*/636 bp	Melon	China: Ningbo/CCYV-nbm	Oct-2009	Gu *et al*. 2011
GU721107	*Hsp70h*/636 bp	Melon	China: Shouguang/CCYV-sgm	Oct-2009	Gu *et al*. 2011
GU721105	*Hsp70h*/636 bp	Melon	China: Shanghai/CCYV-sgm	Oct-2009	Gu *et al*. 2011
HM581659	Complete *Hsp70h*/1671 bp	Cucumber	China: Ninbo/CCYV-nbc	Oct-2009	Gu *et al*. 2011
GU721110	*HSP70h*/636 bp	Cucumber	China: Ninbo/CCYV-nbc	Oct-2009	Gu *et al*. 2011
GU721108	*HSP70h*/636 bp	Watermelon	China: Ninbo/CCYV-nbw	Oct-2009	Gu *et al*. 2011
GU721106	*HSP70h*/636 bp	Watermelon	China: Shouguang/CCYV-sgw	Oct-2009	Gu *et al*. 2011
AB457591	*HSP62h*/472 bp	Melon	Japan: Kumamoto/	2004	Gyoutoku *et al*. 2009
JF807053	*CP*/750 bp	Melon	Sudan/CCYV	Jun-2010	Hamed *et al*. 2011
HM581658	*CP*/753 bp	Cucumber	China: Ninbo/CCYV-nbc	Oct-2009	Gu *et al*. 2011
HM581657	Complete *CPm*/1425 bp	Cucumber	China: Ninbo/CCYV-nbc	Oct-2009	Gu *et al*. 2011

Results

Specific primers for CCYV detection

A 733 bp nucleotide fragment was produced with primers CCYV-jF2 and CCYV-jR2 designed based on CCYV coat protein gene (CP) for RT-PCR. When compared with CCYV-F/R (for *HSP70h*) for RT-PCR amplification, both pairs had equivalent sensitivity to detect up to 10^{-4} dilution of the saps of CCYV-infected melon fruit; however, CCYV-jF2/jR2 had a negative reaction with the health control in contrast to CCYV-F/R which had a nonspecific reaction as shown in Fig. 1.

Full length sequencing of RNA2

Full length sequencing of RNA2 of CCYV-Erlun and CCYV-Yilan were completed by RT-PCR and 5'-RACE. A total of 8041 bp including genes of *p4.9* (1035−1166 nt), *HSP70h* (1207−2877 nt), *p6* (2878−3042 nt), *p59* (3036−4589 nt), *p9* (4571−4810 nt), *CP* (4941−5693 nt), *CPm* (5693−7117 nt), *p26* (7179−7820 nt), and 5'- and 3'-terminal untranslated regions were obtained and submitted to NCBI GenBank. The accession numbers were assigned as JN126045 for CCYV-Erlun and JN126046 for CCYV-Yilan.

Analysis of phylogenetic relationships

Alignment with the relevant nucleotide sequences in the NCBI GenBank, CCYV-Erlun (JN126045) and CCYV-Yilan (JN126046) both had > 99% nucleotide identities to other isolates (Tables 3−6) and their phylogenetic dendrograms were shown in Fig. 2. Results of comparative analyses of nucleotide sequences of CCYV isolates derived from various origins revealed the nucleotide identities as high as 99% to 100% in genes of *HSP70h* (Table 3), *CP* (Table 4) , *CPm* (Table 5) and RNA2 (Table 6). In contrast, they shared 70.1%, 63.6%, 50.6%, and58.2% identities with the above-mentioned corresponding gene sequences of *Cucurbit yellow stunting disorder virus* (CYSDV).

The variability of nucleotide sequences of CCYV RNA2 among a Japan isolate (AB523789) and two Taiwan isolates (JN126045, JN126046) were revealed by Entropy (Hx) plot as shown in Fig. 3. The divergence of nucleotide sequences mostly located in the 5'- and 3'-terminal untranslated regions; otherwise, very low entropies were observed in locations of *HSP70h* (1 nt), *CP* (2 nt) and *CPm* (2nt). Within two Taiwan indigenous isolates of CCYV, only three nucleotides were found differed from each other of entire RNA2 (8041nt) and they were scattered in *CP*, *CPm*, and 3'-terminal regions, respectively.

Discussion

In case of CCYV infection, cucurbit plants always show the typical chlorotic yellowing symptoms on the leaves which affect the yield and fruit quality. The viral disease generally is untreatable with chemicals; thus, the strategy to prevent virus infection and technique to detect the causal agent in advance become relatively important. RT-PCR is a usual technique for RNA virus detection and the heat shock protein-like gene (*HSP70h*) is family-specific to members of *Closteroviridae* (Karasev 2000). Accordingly, primer pair (CCYV-F/R) targeting *HSP70h* (Okuda *et al*. 2010) were used for RT-PCR amplification in this study. However, the *HSP70h* gene in members of *Crinivirus* is highly conserved (Dolja *et al*. 2006) and that lacks of specificity to differentiate the species in this genus by RT-PCR using primers of CCYV-F/R. Other than *HSP70h* gene, a pair of CP-based primers (CCYV-jF2/jR2) were designed and used in RT-PCR to compare the efficiency of both tactics in CCYV detection. Results shown in Fig. 1 demonstrated the RT-PCR products made from CCYV-jF2/jR2 were comparatively denser and clearer than those from CCYV-F/R and as well, no noise interference with healthy control was observed. Consequently, primer pair CCYV-jF2/jR2 for RT-PCR is conducive to subsequent CCYV detection RT-PCR for higher sensitivity and accuracy.

Two isolates of *Cucurbit chlorotic yellows virus*, CCYV-Erlun and CCYV-Yilan, were recovered from melon plants in Taiwan, and their complete RNA2 nucleotide sequences of 8041 bp were determined. Regardless of geographic distributions (Japan, China, Taiwan, and Sudan) and host origins (melon, oriental melon, cucumber, and watermelon), all nucleotide sequences of CCYV isolates tested were closely clustered in phylogenetic tree as shown in Fig. 2 while they were significantly distinctive from other species of *Crinivirus*.

Although CCYV is prevalent in cucurbit production areas, there are only three complete nucleotide sequences of RNA2 available in NCBI GenBank database; they are two Taiwan isolates (JN126045, JN126046) and one Japan isolate (AB523789). By

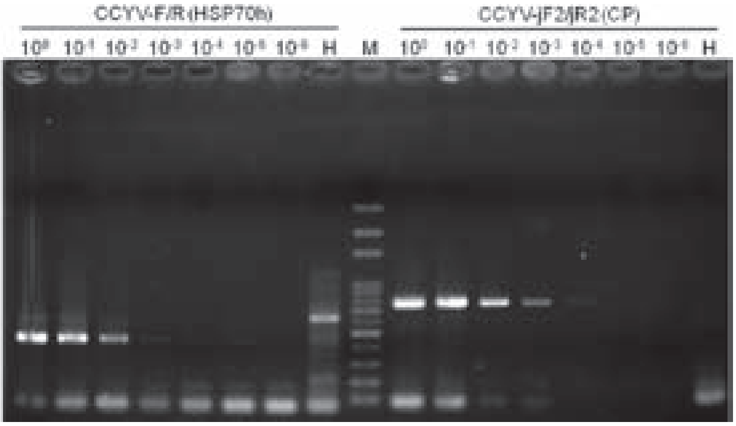

Fig. 1. Comparison of the sensitivity and specificity of the primers, CCYV-F/R (for *HSP70h*) and CCYV-jF2/jR2 (for *CP*) used in RT-PCR for the detection of *Cucurbit chlorotic yellows virus*. Both pairs had equivalent sensitivity to detect up to 10^{-4} dilution of the saps of infected melon fruit. CCYV-jF2/jR2 was negative to the health control in contrast to CCYV-F/R which had a nonspecific reaction to health control.

Table 3. The nucleotide sequence identities of heat shock protein 70-kDa homologue gene (*Hsp70h*) among currently existing *Cucurbit chlorotic yellows virus* (CCYV) isolates

Accession no.of nucleotide [z]		Nucleotide sequence identity (%) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
AB523789	1																	
HQ148667	2	100																
JN126045	3	100	100															
JN126046	4	100	100	100														
JF502222	5	100	100	100	100													
AB457591	6	100	100	100	100	100												
GU721111	7	100	100	100	100	100	100											
JF807055	8	100	100	100	100	100	100	100										
HM581659	9	100	100	100	100	100	100	100	100									
GU721108	10	100	100	100	100	100	100	100	100	100								
GU721110	11	100	100	100	100	100	100	100	100	100	100							
GU721105	12	100	100	100	100	100	100	100	100	100	100	100						
GU721109	13	100	100	100	100	100	100	100	100	100	100	100	100					
GU721107	14	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6				
GU721106	15	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	100			
FJ380119 [y]	16	74.9	74.9	74.9	74.9	74.9	74.9	74.9	74.9	74.9	74.9	74.9	74.9	74.9	74.5	74.5		
FJ492808 [x]	17	70.1	70.1	70.1	70.1	70.1	70.1	70.1	70.1	70.1	70.1	70.1	70.1	70.1	70.5	70.5	71.5	
NC_001598 [w]	18	45.2	45.2	45.2	45.2	45.2	45.2	45.2	45.2	45.2	45.2	45.2	45.2	45.2	45.5	45.5	45.9	44.9

[z] Details of CCYV nucleotide sequences are listed in Table 2.

[y] FJ380119: Complete RNA2 sequence (8556 bp) of *Lettuce chlorosis virus* (LCV), *Crinivirus*, isolated from *Lactuca sativa* in California, USA (Salem *et al*. 2009) .

[x] FJ492808: Complete RNA2 sequence (7976 bp) of *Cucurbit yellow stunting disorder virus* (CYSDV), *Crinivirus*, isolated from melon in Arizona, USA. in October, 2006.

[w] NC_001598: Complete genome (15480 bp ss-RNA) of *Beet yellows virus* (BYV), *Closterovirus*, isolated from Ukrainian, Russia before 1993 (Agranovsky *et al*. 1994).

Table 4. Coat protein gene (*CP*) nucleotide sequence identities of currently existing *Cucurbit chlorotic yellows virus* (CCYV) isolates

Accession no. of nucleotide[z]		Nucleotide sequence identity (%)							
		1	2	3	4	5	6	7	8
JN126046 (Taiwan: Yilan, Melon)	1								
HM581658 (China: Ningbo, Cucumber)	2	99.8							
JN126045 (Taiwan: Erlun, Muskmelon)	3	99.8	99.7						
JF807053 (Sudan, Melon)	4	99.6	99.4	99.7					
AB523789 (Japan: Kumamoto, Melon)	5	99.8	99.7	99.7	99.4				
JF502222 (Taiwan: Yunlin, Muskmelon)	6	99.8	99.7	99.7	99.4	99.7			
FJ380119 (LCV, *Crinivirus*)	7	73.8	73.7	73.7	73.4	74.0	73.8		
FJ492808 (CYSDV, *Crinivirus*)	8	63.7	63.6	63.6	63.3	63.7	63.7	62.6	
NC_001598 (BYV, *Closterovirus*)	9	36.5	36.6	36.6	36.5	36.5	36.5	35.6	34.1

[z] Details of CCYV nucleotide sequences are listed in Tables 2 and 3.

Table 5. Minor coat protein gene (*CPm*) nucleotide sequence identities of currently existing *Cucurbit chlorotic yellows virus* (CCYV) isolates

Accession No. of Nucleotide[z]		Nucleotide sequence identity (%)						
		1	2	3	4	5	6	7
AB523789 (Japan: Kumamoto, Melon)	1							
JF502222 (Taiwan: Yunlin, Muskmelon)	2	100.0						
JN126045 (Taiwan: Erlun, Muskmelon)	3	99.9	99.9					
HM581657 (China: Ningbo, Cucumber)	4	99.9	99.9	100.0				
JN126046 (Taiwan: Yilan, Melon)	5	99.8	99.8	99.9	99.9			
FJ380119 (LCV, *Crinivirus*)	6	64.7	64.7	64.7	64.7	64.6		
FJ492808 (CYSDV, *Crinivirus*)	7	50.8	50.8	50.7	50.7	50.7	50.6	
NC_001598 (BYV, *Closterovirus*)	8	34.0	34.0	33.9	33.9	33.9	33.0	32.0

[z] Details of CCYV nucleotide sequences are listed in Tables 2 and 3.

Table 6. Complete genomic RNA2 nucleotide sequence identities of currently existing *Cucurbit chlorotic yellows virus* (CCYV) isolates

Accession No. of Nucleotide[z]		Nucleotide sequence identity (%)					
		1	2	3	4	5	6
JN126045 (Taiwan: Erlun, Muskmelon)	1						
JN126046 (Taiwan: Yilan, Melon)	2	99.9					
AB523789 (Japan: Kumamoto, Melon)	3	99.9	99.9				
JF502222 (Taiwan: Yunlin, Muskmelon)	4	99.9	99.9	99.9			
FJ380119 (LCV, *Crinivirus*)	5	68.3	68.3	68.4	68.3		
FJ492808 (CYSDV, *Crinivirus*)	6	58.2	58.2	58.2	58.2	57.2	
NC_001598 (BYV, *Closterovirus*)	7	24.4	24.4	24.4	24.4	24.3	23.9

[z] Details of CCYV nucleotide sequences are listed in Tables 2 and 3.

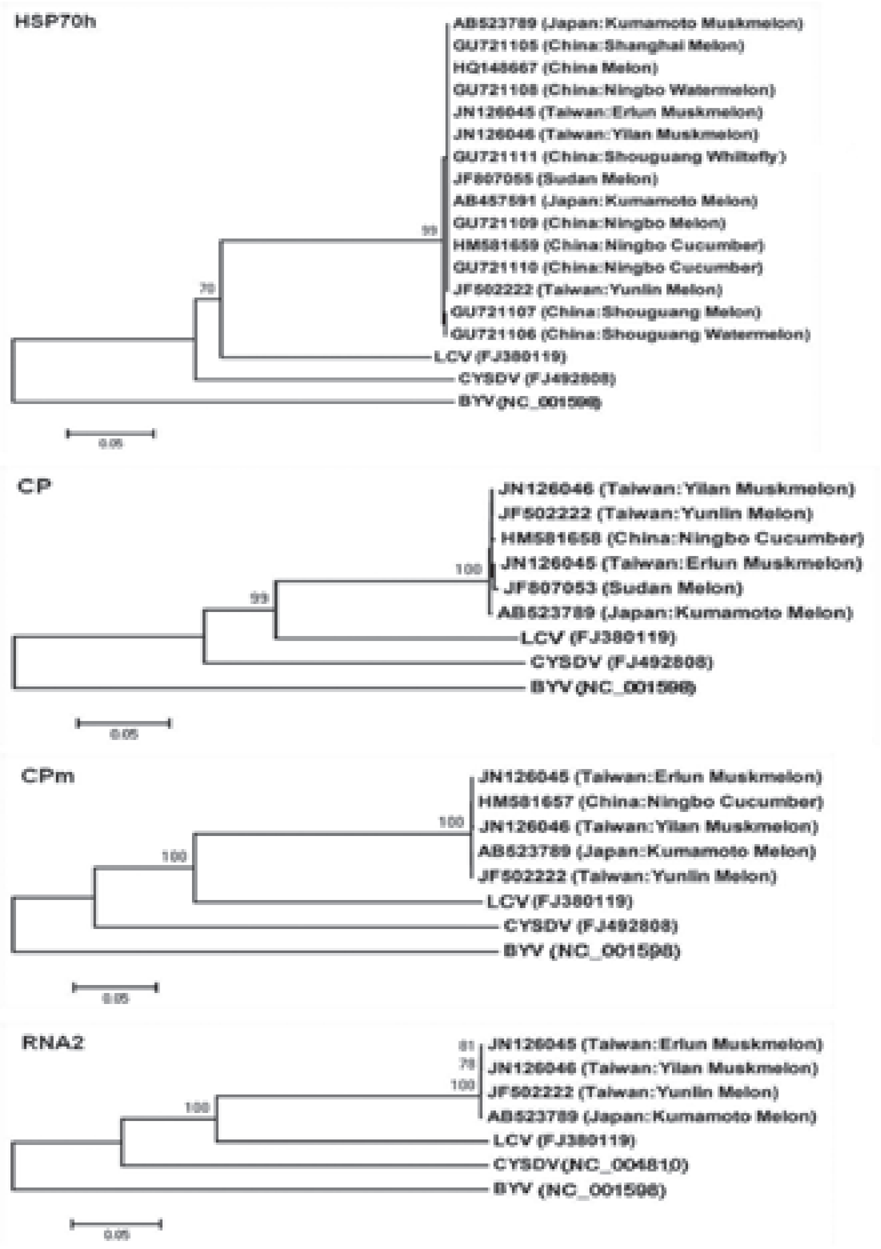

Fig. 2. Phylogenetic dendrograms of respective genes of heat shock protein 70-kDa homologue (*HSP70h*), coat protein (*CP*), minor coat protein (*CPm*) and RNA2 of *Cucurbit chlorotic yellows virus*. Each tree was generated by neighborhood-joining method (MEGA4). Horizontal branch length is proportional to the estimated number of nucleotide substitutions, while boot strap probabilities (shown as percentage) were indicated above or below the internal branches, only values greater than 70% are shown. GenBank accession numbers of the nucleotide sequences used in the phylogenetic analyses are shown and their details are listed in Tables 2 and 3. In addition, CYSDV (NC_004810) is a complete RNA2 sequence of *Cucurbit yellow stunting disorder virus*, Crinivirus, isolated from melon in Spain (Aguilar *et al*. 2003); and BYV (NC_001598) is a closterovirus, set as an outgroup.

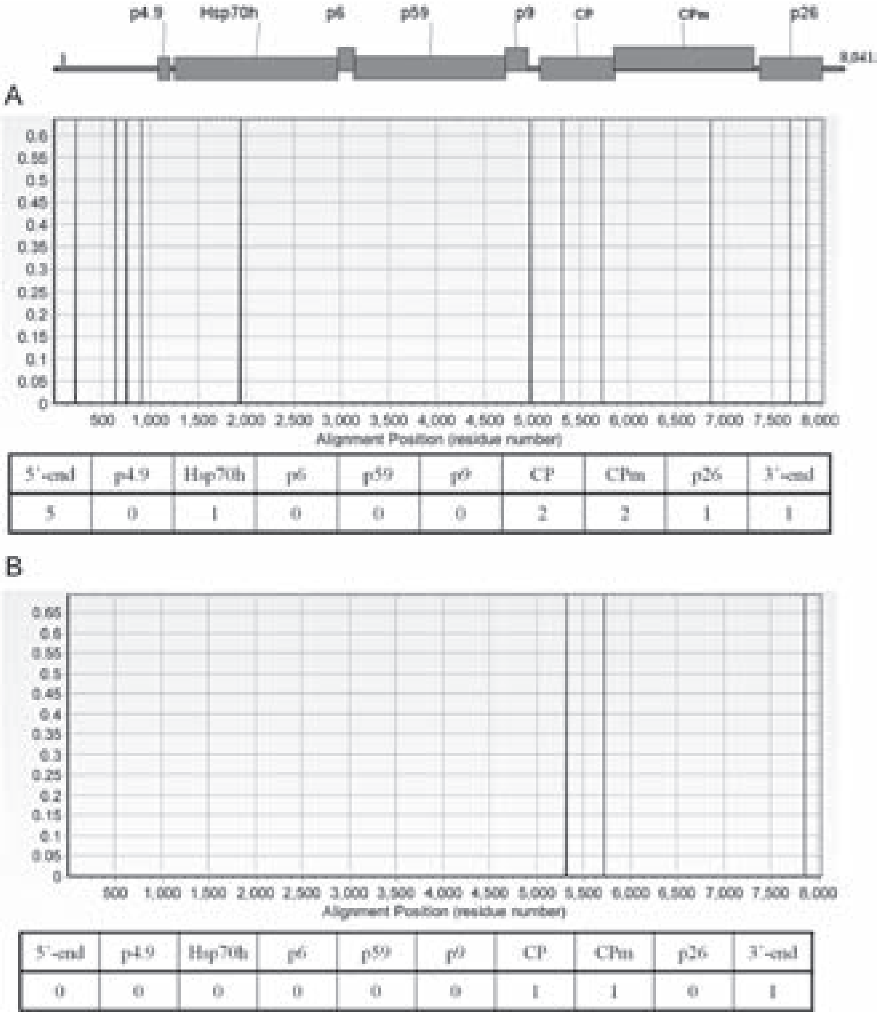

Fig. 3. The entropy (Hx) plot generated with BioEdit v 7.053 showing the nucleotide sequence variability among two Taiwan isolates (JN12604, JN126046) and a Japan isolate (AB523789) of *Cucurbit chlorotic yellows virus* RNA2 (A), and the difference between two Taiwan isolates (B). On the top, the diagram represents the genomic organization of CCYV RNA2; and the numbers of different nucleotides are shown at the bottom of each diagram in corresponding to the names of respective ORFs.

alignment of the entire sequence of RNA2 with 8041 nt, only 12 nt were different as recognized by BioEdit and the major sequence divergences were located in the 5' and 3' end non-translated regions shown by entropy plots (Fig. 3). The sequence variability between the two indigenous Taiwan isolates was localized in 3 divergent nucleotides located respectively in regions of *CP*, *CPm*, and 3'-end. The remarkable homogeneity and extremely close cluster in phylogenetic trees (Fig. 2) of all CCYV nucleotide sequences tested indicate that all the currently existing CCYV isolates are likely derive from the same origin which was found in Kumamoto Prefecture, Kyushu, Japan.

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台灣瓜類退綠黃化病毒 RNA2 之全長核苷酸序列及其與現有株系的比較 [1]

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摘　要

林羿廷、廖吉彥、蔡錦慧、鄧汀欽。2012。台灣瓜類退綠黃化病毒 RNA2 之全長核苷酸序列及其與現有株系的比較。台灣農業研究 61:132–143。

瓜類退綠黃化病毒 (*Cucurbit chlorotic yellows virus*, CCYV) 雲林縣二崙鄉分離株 (CCYV-Erlun) 與宜蘭縣壯圍鄉分離株 (CCYV-Yilan)，以反轉錄聚合酶鏈式反應 (RT-PCR) 及 Rapid amplification of 5’ complementary DNA ends (5’ RACE) 等方法將其 RNA2 完全解序，各得全長為 8041 bp 之核苷酸序列；除 5’- 和 3’- 未轉譯端外，包括 *p4.9* 基因 (132 nt)、*HSP70h* 基因 (1671 nt)、*p6* 基因 (165 nt)、*p59* 基因 (1554 nt)、*p9* 基因 (240 nt)、*CP* 基因 (753 nt)、*CPm* 基因 (1425 nt) 及 *p26* 基因 (642 nt)。利用其中 CP 基因序列設計引子對進行 RT-PCR，可提高 CCYV 檢測的敏感性與準確性。藉由 RNA2 核苷酸序列比對及親緣分析，結果顯示於台灣發現之CCYV-Erlun (JN126045) 及CCYV-Yilan (JN126046) 都與日本、台灣、中國及蘇丹之 CCYV 相關核苷酸序列相同度達 99–100%。熵圖 (Entropy plot) 顯示兩個台灣分離株與日本原始分離株的 RNA2 核苷酸差異只有 12 nt (在總數 8041 nt 中)，且主要都發生在 5’- 及 3’- 端未轉譯區。本研究發現目前全球各地發生之 CCYV 分離株彼此間基因同質性極高，親緣關係非常近，因此推論這些病毒都是同一來源。

關鍵詞：瓜類退綠黃化病毒、反轉錄聚合酶鏈式反應、親緣分析、熵圖。

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台灣農業研究 (J. Taiwan Agric. Res.) 61(2):144–157 (2012)

臺潛蠅繭蜂 (*Opius caricivorae*) (膜翅目：小繭蜂科) 之形態與生活史[1]

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摘　　要

錢景秦、張淑貞。2012。臺潛蠅繭蜂 (*Opius caricivorae*) (膜翅目：小繭蜂科) 之形態與生活史。台灣農業研究 61:144–157。

臺潛蠅繭蜂 (*Opius caricivorae* Fischer) 行單產雄性孤雌生殖、單員幼蟲至蛹內寄生。交尾與否對雌蜂生育力無影響。雄蜂一生有多次交尾 (8–17 次)，雌蜂多數只交尾 1 次，僅 4.5% 交尾 2–4 次。交尾前期為 1.1 小時。該蜂有取食寄主現象，卻不致死寄主。羽化盛期為上午 5 點至 8 點，產卵盛期為上午 5 點至 9 點。該蜂發育不受寄主齡期影響。在 25℃ 下，卵至蛹期發育所需時間為 14.9–15.0 日，其中卵期為 2.2 日，幼蟲期為 5.0–5.1 日，前蛹期為 1.6–1.8 日，蛹期為 5.9–6.1 日。卵發育至蛹期之存活率為 86.5–91.6%。每日供應 40–50 隻第三齡蔬菜斑潛蠅 (*Liriomyza sativae* Blanchard) 幼蟲時，雌、雄蜂壽命各為 21.9 日與 22.8 日，每雌蜂可產 277 隻子蜂，子代雌性比為 0.62。雌蜂產卵數、過寄生率及對寄主之利用率均顯著受寄主蔬菜斑潛蠅密度影響。寄主密度為 1、2 及 5 隻時，雌蜂產卵數少 (4.4–7.6 粒)，過寄生率高 (66.5–87.5%)；寄主密度為 20、30 及 40 隻時，雌蜂產卵數多 (19.1–24.0 粒)，過寄生率低 (0.9–5.5%)。寄主密度為 1、2、5、10 及 20 隻時，雌蜂對寄主之利用率高達 80.0–100%，但寄主密度為 30 隻與 40 隻時，雌蜂對寄主之利用率各達 62.9% 與 59.4%。

關鍵詞：臺潛蠅繭蜂、蔬菜斑潛蠅、外部形態、生活史。

前　　言

臺潛蠅繭蜂 (*Opius caricivorae* Fischer) 屬膜翅目 (Hymenoptera)、小繭蜂科 (Braconidae)、蠅繭蜂屬 (*Opius*)，於 1964 年被定名 (Yu *et al*. 2009)。中文異名為黃腹蠅繭蜂 (Yin *et al*. 2003; Wan *et al*. 2005; Xu *et al*. 2007a, b)，英文異名為 *Opius labradorensis* Fisher (Yu *et al*. 2009)。

該蜂地理分布於西舊北區、歐洲、東舊北區及東方等地 (Yu *et al*. 2009)，包括芬蘭、捷

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克斯洛伐克、德國、奧地利、波蘭、匈牙利、意大利、西班牙、土耳其、阿富汗、烏克蘭、俄羅斯、韓國及中國 (浙江、廣西、雲南、貴州) (Wen *et al*. 2002; Yu *et al*. 2009)。

臺潛蠅繭蜂之食性為寡食性，寄主範圍限雙翅目潛蠅科，如 *Agromyza nigrescens* Hendel、危害苔草菊 (*Carex hirta* L.) 之 *Cerodontha angulata* (Loew) 與 *Cerodontha caricicola* Hering、*Cerodontha caricivora* Groschke、*Cerodontha eucaricis* Nowakowski、危害薊荊 [*Cirsium arvense* (L.)] 之韭潛蠅 [*Chromatomyia horticola* (Goureau)]、危害大蔥 (*Allium fistulosum* L.) 之 *Liriomyza chinensis* Kato、南美斑潛蠅 [*Liriomyza huidobrensis* (Blanchard)]、蔬菜斑潛蠅 (*Liriomyza sativae* Blanchard)、非洲菊斑潛蠅 [*Liriomyza trifolii* (Burgess)]、危害丹參藻 (*Salvia verticillata* L.) 之 *Napomyza salviae* Hering、危害沼澤金盞花 [*Caltha palustris* (Cowslip)] 之 *Phytomyza* sp.、*Phytomyza lappae* Goureau、*Phytomyza lappina* Goureau、危害薊荊之 *Phytomyza spinaciae* Hendel、危害 *Amaranthus lividus* L. 之 *Scaptomyza flava* (Fallen) (Lin & Wang 1992; Chien & Ku 1998; Wen *et al*. 2002; Chen *et al*. 2003; Yu *et al*. 2009)。

寄生方式屬幼蟲至蛹單員內寄生 (Chien & Ku 2001; Yin *et al*. 2003)。產卵方式屬共育寄生 (koinobiont)，偏好在非洲菊斑潛蠅第三齡幼蟲上產卵 (Chien & Ku 2001)。Yin *et al*. (2003) 亦報導成蜂部分習性。臺潛蠅繭蜂在中國浙江省杭州地區為蔬菜斑潛蠅重要的寄生蜂 (Chen *et al*. 2003)，在台灣該蜂亦寄生蔬菜斑潛蠅、南美斑潛蠅 (Chien & Chang unpublished data) 及非洲菊斑潛蠅 (Lin & Wang 1992; Chien & Ku 1998)。

為瞭解臺潛蠅繭蜂對蔬菜斑潛蠅之抑制能力，本研究以該蠅為寄主，觀察該蜂之外部形態與生活史，藉以提供該蜂辨識、繁殖方法及生物防治時評估寄生蜂對寄主防治潛能之參考。

材料與方法

供試蟲源

在雲林縣林內鄉菜豆 (*Phaseolus vulgaris* L.) 上，採集被蔬菜斑潛蠅幼蟲危害之葉片，攜回室內。攤開葉片置放在塑膠盤內，待老熟幼蟲鑽出化蛹後，收集蠅蛹於玻璃皿內。待成蠅與臺潛蠅繭蜂羽化，供後續試驗飼育之蟲源。

供試寄主植物、寄主害蟲及寄生蜂之繁殖

本試驗所用之寄主植物菜豆苗 (*Phaseolus vulgaris* var. *communis* Aeschers) 之栽培，與供試寄主蔬菜斑潛蠅之繁殖，係參照 Chien & Ku (1996) 之方法。臺潛蠅繭蜂之繁殖，則參照 Chien & Ku (2001) 之方法，以帶有蔬菜斑潛蠅第三齡幼蟲潛食之罐插菜豆苗，繁殖臺潛蠅繭蜂。

各蟲期之外部形態

在 25℃ 下參照 Chien & Ku (2001) 之方法，將內有 120 隻第三齡蔬菜斑潛蠅幼蟲潛食之罐插菜豆苗 (每葉 30 隻)，放入直徑 20 cm、高 25 cm 之壓克力筒，供 30 隻臺潛蠅繭蜂雌蜂產卵 2 小時 (上午 8 點至 10 點間)，然後將罐插菜豆苗移出。繼之在溫度 25℃、相對濕度 65–85% 及光週期 12 L：12 D (上午 5 點至下午 5 點間照光) 下，每日取樣解剖寄主，在立體顯微鏡 (Wild，接目鏡 20×、接物鏡 5×) 下直接觀察臺潛蠅繭蜂卵、幼蟲、前蛹及蛹期之外部形態，並以立體顯微鏡中之測微尺，直接測量各蟲期之體型大小，及藉幼蟲頭寬判定幼蟲之各齡期，各觀察 17–20 隻及 20 隻。成蟲之外部形態亦在立體顯微鏡下觀察，並以立體顯微鏡中之測微尺，直接測量雌、雄蟲體型大小，各觀察 20 隻；觸角先在 10% KOH 溶液中浸漬 4 小時，再以 75% 酒精略為清洗，隨即以不同濃度酒精 (75、80、90 及 95%) 逐一各脫水 2–3 分鐘，最後以 Euparal 封片、

照相，雌、雄蜂各觀察 20 隻；雌蜂前翅之製作，雌蜂先浸 75% 酒精，繼之在立體顯微鏡下拆解其前翅，並以 75% 酒精略為清洗，隨即以 Euparal 封片並鏡檢、照相。

發育各期之時間與過程

於前項外部形態觀察之試驗中，同時亦每日觀察 25℃ 下該蜂卵、幼蟲及蛹期之存活率與發育日數，並在近各蟲期變化或齡期蛻皮之際，每小時記錄該蜂之發育情形。其中存活率試驗，25–35 粒卵為一組，計 3–4 重複；發育期試驗各觀察 17–42 隻不等。

成蜂行為

羽化：2004 年 9 月在 25℃、相對濕度 65–85% 及自然光照下，觀察室內臺潛蠅繭蜂之羽化方式，並記錄一日內，該蜂每小時之羽化頻率及性比。觀察蟲數為 203 隻。

交尾：交尾方式、時間及交尾前期之觀察，係在前述相同條件下，將單個蛹分別置於直徑 1.5 cm、高 4 cm 之小玻璃管內，待成蜂羽化後，即加以配對，移入另一小玻璃管內，供食未稀釋之蜂蜜，並觀察其交尾方式與時間，進行 43 次，交尾前期之觀察則進行 25 次。成蜂第一、二日交尾率之觀察，此時為餵食蜂蜜之方便性，係將上午 5 點至 11 點間羽化之雌、雄蜂分別釋入直徑 12 cm、高 21 cm 之玻璃筒內，供食未稀釋蜂蜜做群體飼育，待下午 3 點時才將雌、雄蜂各 1 隻移入小玻璃管內加以配對，登錄 1 小時內雌、雄蜂之交尾狀況，其中已交尾者記錄為第一日之交尾對數後移除，剩下未交尾者將雌、雄蜂分離，各自釋入上述相同大小之玻璃筒內、供食未稀釋蜂蜜做群體飼育，待第二日早上 9 點才重新再將前日未曾交尾之雌、雄蜂移入小玻璃管內加以配對，登錄 1 小時內成蜂之交尾狀況，記錄為第二日之交尾對數，共進行 70 對成蜂。雌、雄蜂終生交尾次數之觀察，係將單個蛹分別置於上述之小玻璃管內，待蜂羽化後，每日供食未稀釋之蜂蜜，隔日上午 10–12 點隨機單對雌、雄蜂配對，登錄 10 分鐘內雌、雄蜂之交尾狀況，隨後將雌、雄蜂分離，並依雌、雄蜂之交尾次數分別標示為 1 或 0；第三日將此批已交尾 1 或 0 次之雌、雄蜂與另一批羽化次日未交尾之雌、雄蜂配對，觀察方法如前，10 分鐘後將雌、雄蜂分離，並依雌、雄蜂之交尾次數分別標示為 2 或 1 或 0；如此連續觀察至成蜂死亡，累積記錄雌、雄蜂各自之總交尾次數，共觀察雌蜂 66 隻、雄蜂 43 隻。

產卵與取食寄主：雌蜂產卵方式、產卵時間、取食寄主方式及取食寄主時間等之觀察，係參照 Chien & Ku (2001) 之方法，先準備帶有寄主蔬菜斑潛蠅第三齡幼蟲潛食之罐插菜豆苗，將其置入上述相同大小之壓克力筒，在 25℃下再將已交尾雌蜂釋入，觀察雌蜂之產卵方式、產卵時間、取食寄主方式及取食寄主時間。各進行 10–18 重複。產卵時刻之觀察，則在 25℃、光週期 12 L：12 D (上午 5 點至下午 5 點間照光) 下，自清晨 1 點起至次日凌晨 1 點止，在直徑 12 cm、高 21 cm 玻璃筒內，每隔 4 小時，更換內有 40 隻寄主第三齡幼蟲潛食之罐插菜豆苗，供 1 隻已有產卵經驗之第四、五日齡雌蜂產卵；15 日後，記錄雌蜂於各處理時段內之子蜂數，以估測各處理時段內之產卵數。計 10 重複。

交尾對繁殖力之影響：試驗分二種方式進行，即分別為未交尾雌蜂 1 隻，與終生在一起交尾 1 或 1 次以上之雌、雄蜂 1 對。25℃ 下，將 0 日齡供試蜂置入上述相同大小之接蜂用玻璃筒內，每處理每日供應 1 株內有 40–50 隻寄主第三齡幼蟲潛食之罐插菜豆苗，並以細毛筆將未稀釋蜂蜜塗於玻璃筒內壁，直至雌蜂死亡為止。記錄其間處理組雌蜂之壽命、子蜂數及子代雌性比 [♀/(♀ + ♂)]，各做 11 重複與 12 重複。

寄主密度對雌蜂寄主利用率 (host utilization) 與過寄生 (superparasitism) 之影響

先準備各帶有寄主蔬菜斑潛蠅 1、2、5、10、20、30 及 40 隻第三齡幼蟲潛食之罐插菜豆苗，分別將其置入直徑 12 cm、高 21 cm 之玻璃筒內。在 25℃ 下早上 9 點將第五至第六日齡已有產卵經驗之 1 隻雌蜂釋入，至下午 1 點將雌蜂移出。3 日後，在立體顯微鏡 (Wild，接目鏡 20×、接物鏡 5×) 下解剖紀錄各處理每隻寄主蛹體內之蜂卵數，以估測雌蜂在不同寄主蟲數下對寄主之利用率 (寄生寄主數 ÷ 供試寄主蟲數)，與蜂卵在每一寄主上之分布百分率。各進行 8–10 重複。

統計分析

各項處理之試驗資料利用 SAS-EG (SAS Enterprise Guide) 4.1 版統計分析軟體先進行變方分析 (analysis of variance, ANOVA)，再以最小顯著差異性 (least significant difference, LSD) 測驗，在 5% 顯著水準下比較處理間平均值之差異。若遇百分率時，資料先進行角度轉換 (arcsine transformation)，再進行分析。

結　　果

各蟲期之外部形態

成蜂：雌 (圖 1A)，體亮黑色。複眼黑色，觸角柄節、唇基、口器及足黃褐色，觸角梗節、鞭節褐色，前伸腹節、腹部第一、二節背板黃褐色。頭部光滑。觸角絲狀，其長約為體長之 1.3 倍，鞭節具 20–23 節，以 22 節者占 60% (n = 20，圖 2A)。唇基半圓形，無口窩。前、中後胸背板光滑，前伸腹節與腹部第一節有皺紋。前翅翅痣楔形；徑脈 (r1, 1st abscissa radius) 由翅痣近基部 1/3 處發出，徑脈第二段 (r2, 2nd abscissa radius) 長約為橫肘脈 (Cuqu1, 1st transverse cubitus) 的 2.0 倍，徑室 (R, radial cell) 達翅尖旁，徑脈第三段 (r3, 3rd abscissa radius) 長約為徑脈第二段 (r2) 的 2.4 倍；亞盤脈 (n-par, nervus parallelus) 由臀室 (B, brachial cell) 近中部發出，臀室下角關閉不全 (圖 2B)。腹部第一背板呈梯形，其長與寬部略等，其前方 1/3 處具 2 斜伸的脊，其間具皺紋，腹部其餘部分光滑。產卵管微露出腹末。雄蜂形態特徵與雌蜂相同，觸角絲狀，鞭節具 19–23 節，以 21 節者占 45% (n = 20，圖 2C)，翅脈圖與雌相同。雌成蟲體型大於雄成蟲 (表 1)。

卵：呈 hymenopteriform type，透明、無色。蜂卵之大小隨發育而變大 (表 1)，初產時呈圓柱形 (cylindrical) (圖 1B)，近孵化時呈卵形 (obovate) (圖 1C)。

幼蟲：寄生蜂幼蟲共有 3 個齡期。各齡頭寬與大小見表 1。第一齡幼蟲為尾顎型 (caudate-mandibulate type)，體透明、無色，頭寬大、長方形、初孵化時其長度約占全身之 1/3 強，大顎明顯，其餘體節寬度逐節縮小，但至第一齡末時，頭部長度約占全身之 1/4，頭與大顎呈淡褐色，消化管內有少許黃色內容物 (圖 1D)。第二齡幼蟲為 hymenopteriform type，體透明、無色，略呈紡錘形，消化管內有橙黃色內容物 (圖 1E)，第三齡幼蟲體黃色，呈長紡錘形，體表密佈短毛，腹部消化管內容物呈紅褐色 (圖 1F)。

前蛹：體黃色 (圖 1G)。體形大小見表 1。

蛹：體黃色、複眼與單眼紅色 (圖 1H)。體形大小見表 1。

發育各期之時間與過程

寄主齡期對臺潛蠅蔺蜂之發育日與存活率均無影響 (表 2、3)。該蜂可產卵於蔬菜斑潛蠅第一至三齡幼蟲體內。在 25℃ 下卵發育至蛹期需時 14.9–15.0 日。其中卵期為 2.2 日；幼蟲期為 5.0–5.1 日，第一至三齡幼蟲發育所需時間各為 1.2、0.6 及 3.2 日；前蛹期為 1.6–1.8 日；蛹期為 5.9–6.1 日。卵發育至蛹期之存活率為 86.5–91.6%，其中卵、前蛹及

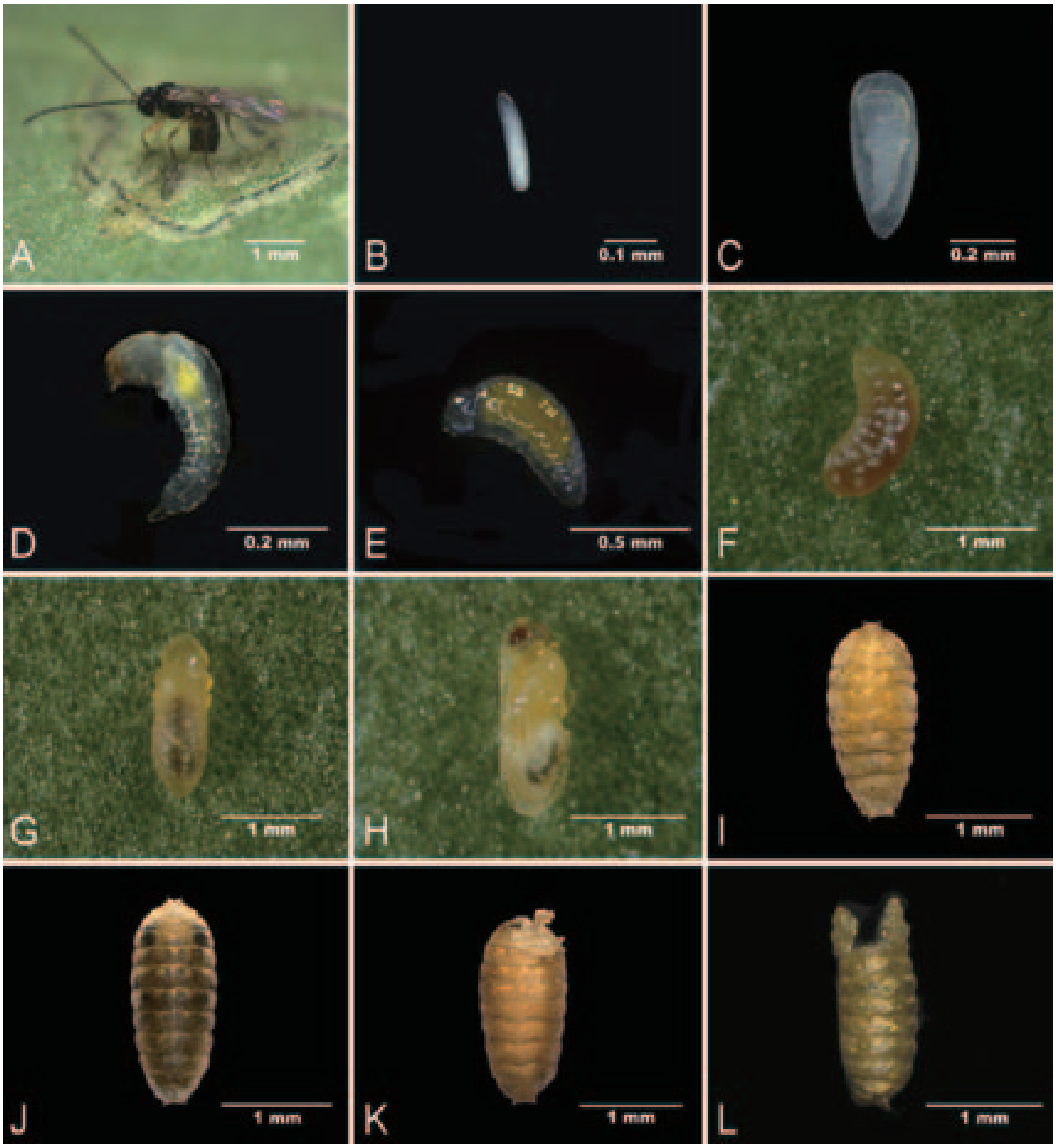

圖 1. 臺潛蠅繭蜂之各生長期外觀 (A–H) 與被寄生蔬菜斑潛蠅蛹之辨識 (I–L)。(A) 產卵寄生中之雌蜂；(B) 卵 (產卵後 2 小時)；(C) 卵 (產卵後 52 小時)；(D) 第一齡幼蟲；(E) 第二齡幼蟲；(F) 第三齡幼蟲；(G) 前蛹；(H) 蛹；(I) 被寄生後之蔬菜斑潛蠅蛹；(J) 正常之蔬菜斑潛蠅蛹；(K) 寄生蜂羽化孔；(L) 蔬菜斑潛蠅羽化孔。

Fig. 1. Morphology of different developmental stages of *Opius caricivorae* (A−H) and characteristics of pupae of leafminer *Liriomyza sativa* parasitized by *O. caricivorae* (I−L). (A) a female wasp parasitizing a leafminer; (B) egg (2 h after oviposition); (C) egg (52 h after oviposition); (D) 1st instar larva; (E) 2nd instar larva; (F) 3rd instar larva; (G) prepupa; (H) pupa; (I) host pupa parasitized by wasp; (J) normal host pupa; (K) emergence hole of wasp; (L) emergence hole of host leafminer.

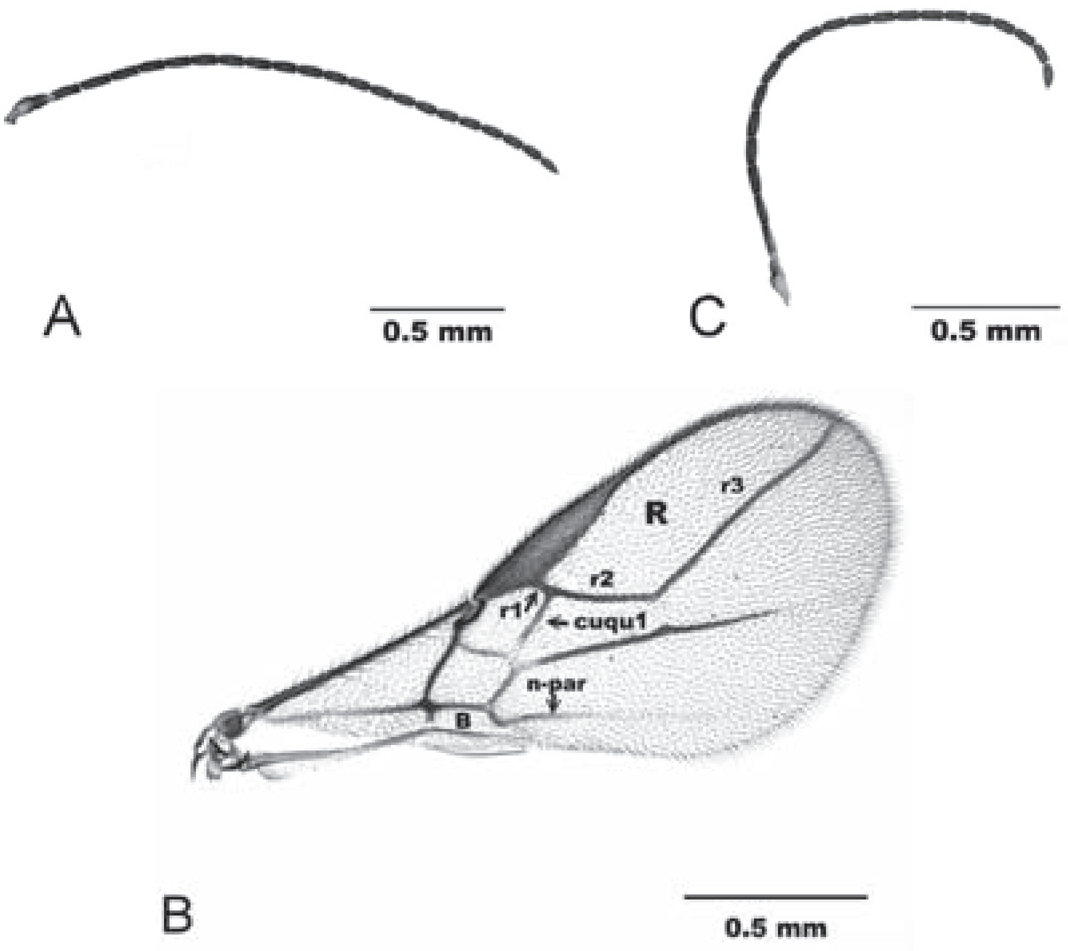

圖 2. 臺潛蠅繭蜂之觸角與前翅。(A) 雌蜂觸角；(B) 雌蜂前翅；(C) 雄蜂觸角。

Fig. 2. Antenna and fore wing of *Opius caricivorae*. (A) female antenna; (B) female fore wing; (C) male antenna. B, brachial cell; cuqu1, 1st transverse cubitus; n-par, nervus parallelus; r1, 1st abscissa radius; r2, 2nd abscissa radius; r3, 3rd abscissa radius; and R, radial cell.

蛹期之存活率均為100%，幼蟲期之存活率為86.5–91.6%。

蜂卵產在寄主幼蟲體內，卵未被包囊 (encapsulation)。卵初產時長度僅 0.18 mm、寬度 0.06 mm，但至 18 小時後，卵開始持續膨大，至 52 小時 (近孵化時) 卵之長度與寬度已各增長 2.6 倍與 3.2 倍 (表 1)。視雌蜂卵產於寄主之齡期，孵化後之第一齡幼蟲，即隨寄主之發育在寄主幼蟲、或蛹體內取食，至第三齡幼蟲，食量大增，約 23 小時即將寄主蛹體液完全吸盡，並緊依寄主蛹壁吐絲做一薄繭，此時幼蟲靜止不動全身緊縮、隨之排出一坨褐色排泄物，進入前蛹。經 1.6–1.8 日變為蛹。再經 5 日蛹體由淡褐色變黑，次日羽化為成蜂。若遇過寄生時，卵雖均可孵化，但僅 1 隻幼蟲順利發育，其餘均在第一齡幼蟲期死亡。當寄生蜂在卵與幼蟲期時，被寄生之蔬菜斑潛蠅蛹與正常之蔬菜斑潛蠅蛹在外形上無法分辨，直至

表 **1.** 臺潛蠅繭蜂寄生蔬菜斑潛蠅時各蟲期體型之大小

Table 1. Body size of the various stages of wasp *Opius caricivorae* on the host leafminer *Liriomyza sativae* [z]

	Head width (mm) of wasp		Body size (mm, Mean ± SEM) of wasp		
Stages of wasp	*n*	Mean ± SEM [y]	*n*	Length	Width
Egg					
Early			20	0.18 ± 0.00	0.06 ± 0.00
Late			17	0.47 ± 0.01	0.19 ± 0.00
Larva					
1st	20	0.18 ± 0.00			
Early			20	0.57 ± 0.01	0.19 ± 0.00
Late			20	0.71 ± 0.01	0.22 ± 0.00
2nd	20	0.23 ± 0.00			
Early			20	0.75 ± 0.00	0.27 ± 0.00
Late			20	1.12 ± 0.00	0.43 ± 0.00
3rd	20	0.28 ± 0.00			
Early			20	1.20 ± 0.01	0.52 ± 0.00
Late			20	1.47 ± 0.01	0.55 ± 0.00
Prepupa			17	1.52 ± 0.00	0.63 ± 0.02
Pupa			17	1.61 ± 0.03	0.62 ± 0.01
Adult					
Female			20	1.65 ± 0.01	0.41 ± 0.01
Male			20	1.58 ± 0.02	0.39 ± 0.01

[z] The experiment was conducted at 25℃.
[y] SEM: standard error of mean.

表 **2.** 蔬菜斑潛蠅幼蟲齡期對臺潛蠅繭蜂卵、幼蟲、前蛹、蛹及卵至蛹期發育日數之影響

Table 2. Effect of instars of *Liriomyza sativae* on duration (d) of egg, larval, prepupal, pupal and egg to pupal stages of *Opius caricivora* [z]

	Duration (d) of the stages of *Opius caricivorae*									
Instars of host *L. sativae*	Egg		Larva		Prepupa		Pupa		Egg to pupa	
	n	Mean ± SEM [y]	*n*	Mean ± SEM	*n*	Mean ± SEM	*n*	Mean ± SEM	*n*	Mean ± SEM
1st	33	2.2 ± 0.0 a	33	5.1 ± 0.2 a	30	1.8 ± 0.3 a	30	5.9 ± 0.3 a	30	15.0 ± 0.3 a
2nd	20	2.2 ± 0.0 a	20	5.0 ± 0.1 a	17	1.6 ± 0.1 a	17	6.1 ± 0.1 a	17	14.9 ± 0.3 a
3rd	42	2.2 ± 0.0 a	42	5.0 ± 0.0 a	37	1.7 ± 0.2 a	37	6.1 ± 0.2 a	34	15.0 ± 0.2 a

[z] The experiment was conducted at 25℃.
[y] SEM: standard error of mean. Means within each column followed by the same letter are not significantly different at 5% level by LSD test.

寄生蜂發育至前蛹時，因老熟幼蟲之吐絲做繭致使被寄生之蔬菜斑潛蠅蛹在照光下，外觀上除可看到較具光澤之寄主蛹殼外，亦可隱約透見其內寄生蜂前蛹之外廓 (圖 1I)，而此時正常之蔬菜斑潛蠅蛹已發育至近羽化前，透過斑潛蠅蛹殼可明顯看到其內斑潛蠅黑色的 2 個複眼 (圖 1J)。

成蜂行為

羽化：蜂蛹發育成熟後，成蜂在靠近蔬菜斑潛蠅蛹殼頂端之上側面咬一長 0.60 mm ±

表 3. 蔬菜斑潛蠅幼蟲齡期對臺潛蠅繭蜂卵、幼蟲、前蛹及卵至蛹期存活率之影響

Table 3. Effect of instars of *Liriomyza sativae* on survival of egg, larval, prepupal, pupal, and egg to pupal stages of *Opius caricivora* [z]

Instars of host	Survival (%) of the stages of *Opius caricivora*				
L. sativae	Egg	Larva	Prepupa	Pupa	Egg to pupa
1st	100.0 ± 0.0 a [y]	91.6 ± 2.3 a	100.0 ± 0.0 a	100.0 ± 0.0 a	91.6 ± 2.3 a
2nd	100.0 ± 0.0 a	86.5 ± 3.7 a	100.0 ± 0.0 a	100.0 ± 0.0 a	86.5 ± 3.7 a
3rd	100.0 ± 0.0 a	89.0 ± 3.6 a	100.0 ± 0.0 a	100.0 ± 0.0 a	89.0 ± 3.6 a

[z] The experiment was conducted with three to four replicates at 25℃, 25–35 eggs/treatment.

[y] Mean (Y) ± standard error. Means within each column followed by the same letter are not significantly different at 5% level by LSD test. Data were arcsin-square-root of (1-Y) transformed prior to analysis.

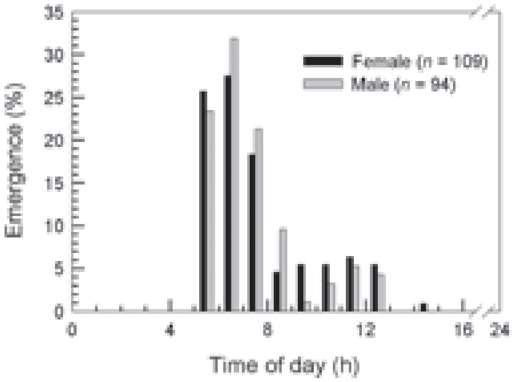

圖 3. 在 25℃下臺潛蠅繭蜂之日羽化時刻。

Fig. 3. Daily emergence rhythm of *Opius caricivorae* at 25℃.

0.01 mm、寬 0.46 mm ± 0.03 mm 之羽化洞鑽出 (*n* = 20，圖 1K)，與未被寄生蔬菜斑潛蠅之羽化孔 (圖 1L) 不一。羽化時刻為上午 5 點至下午 3 點之間，但 73.9% 集中於上午 5 點至 8 點 (*n* = 203，圖 3)。雄蜂與雌蜂羽化時刻略同。具趨光性。成蜂羽化後先排出一小段黑色排泄物，再利用左、右後足之脛節與跗節夾住原先縐縮之翅，不斷梳理，其中 86.7% 者 (*n* = 30) 完成展翅需時 11.2 分鐘 ± 0.9 分鐘，另 13.3% 者則需時 30.8 分鐘 ± 2.3 分鐘。

交尾：92.9% 蜂羽化當日即可交尾，但亦有 7.1% 蜂延至次日上午完成交尾 (*n* = 70)。羽化當日交尾成蜂之交尾前期為 1.1 小時 ± 0.1 小時 (11–148 分鐘，*n* = 25)。交尾時雄蜂揮動觸角尋找雌蜂，觸及雌蜂後即爬上其背，然後身軀退後，彎曲腹部與雌蜂交尾。每次交尾時間需 28.8 秒 ± 1.9 秒 (11–65 秒，*n* = 43)。95.5% 雌蜂一生交尾 1 次，僅 4.5% 者交尾 2–4 次 (*n* = 66)，而雄蜂一生交尾次數則多達 8–17 次 (*n* = 43)。

產卵：產卵時雌蜂在豆葉上一面行走，一面揮動觸角尋找潛食在豆葉內之寄主蔬菜斑潛蠅，若探觸及寄主幼蟲時，雌蜂即下壓腹部將產卵管刺向豆葉內之寄主，並將卵產下。產卵需時 21.7 秒 ± 1.1 秒 (16–30 秒，*n* = 18)。產卵時刻以上午 5 點至 9 點間產卵數最多 (16.3 粒，占 70.3%)，其次為上午 9 點至下午 1 點 (4.2 粒，占 18.1%) 與下午 1 點至 5 點間 (2.3 粒，占 9.9%)，再次為下午 5 點至 9 點 (0.4 粒，占 1.7%)、下午 9 點至凌晨 1 點 (0.0 粒，占 0.0%) 及凌晨 1 點至 5 點 (0.0 粒，占 0.0%) (*n* = 10) (圖 4)。寄生寄主方式為單員內寄生，雌蜂在 1 隻寄主上僅產 1 粒卵，但若在蜂多、寄主少時，1 隻寄主體內之蜂卵曾多達 9 粒。雌蜂產卵時，寄主幼蟲停滯不取食，但待雌蜂產完卵離開後，該被寄生之寄主恢復正常取食並發育至蛹。

取食寄主：雌蜂有取食寄主行為，但不致死寄主。取食寄主時，雌蜂先藉產卵管透過豆葉鑽刺寄主蔬菜斑潛蠅幼蟲之體表，再以口器在該鑽刺處取食寄主幼蟲之體液，此鑽刺與吸

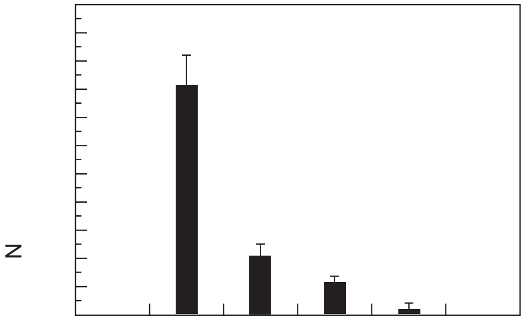

圖 4. 臺潛蠅繭蜂雌蜂在 25℃與光週期 (上午 5 點至下午 5 點間照光) 下平均日產卵時刻。

Fig. 4. Daily oviposition rhythm of female *Opius caricivorae* in a growth chamber at 25℃ under 12 h light (5:00 a.m. to 17:00 p.m.). Error bars represent standard error (n = 10). Different letters above average values indicate significant differences at $P < 0.05$ according to LSD test.

食動作快速重複為之，但偶有僅見鑽刺行為卻未有連續之取食動作。75.0% 雌蜂 (n = 16)，每次取食寄主需 2.7 分鐘 ± 0.3 分鐘，其中鑽刺寄主動作與吸食寄主動作，各為 23.0 次 ± 3.2 次與 22.0 次 ± 3.1 次；另 25.0% 雌蜂每次取食寄主需長達 6.2 分鐘 ± 0.7 分鐘，其中鑽刺寄主動作與吸食寄主動作，各為 63.8 次 ± 3.5 次與 63.0 次 ± 3.9 次。雌蜂取食寄主時，寄主幼蟲停滯不取食，但待雌蜂取食完離開後，該被取食之寄主恢復正常取食並發育至蛹。

交尾對繁殖力之影響：生殖方式為單產雄性孤雌生殖 (arrhenotoky)。未交尾雌蜂之壽命與子蜂數，與交尾者均無顯著差異，二者壽命各為 19.3 日與 21.9 日，子蜂數各為 213 隻與 277 隻；子代雌性比方面二者呈顯著差異，未交尾者為 0，而交尾者為 0.62 (表4)。

寄主密度對雌蜂寄主利用率與過寄生率之影響

寄主利用率：雌蜂對寄主之利用率顯著受寄主蔬菜斑潛蠅密度之影響。寄主密度為 2 隻與 20 隻，1、5 及 10 隻，及 30 隻與 40 隻時，雌蜂對寄主之利用率各達 100% 與 90.0%，80.0–86.1% 及 62.9% 與 59.4% (表 5)。

過寄生率：雌蜂產卵數與過寄生率均顯著受寄主蔬菜斑潛蠅密度之影響。雌蜂產卵總數在寄主密度 40 隻時最多 (24.0 粒)，其次為寄主密度 20 隻與 30 隻時 (19.1 粒與 19.9 粒)，寄主密度 1、2、5 及 10 隻時，雌蜂之產卵總數僅 4.4–9.0 粒 (表 6)。至於每一寄主上之蜂卵數，在寄主密度 1、2、5 隻時較多，該寄主上之蜂卵數最多者可各達 9、6 及 5 粒，此時雌蜂過生率高達 66.5–87.5%；寄主密度 10、20、30 及 40 隻時每一寄主上之蜂卵數較少，該寄主上之蜂卵數最多者僅各達 3、2、2 及 2 粒，此時雌蜂過生率甚低，達 0.9–9.2%。

討　論

行內寄生生活方式之寄生蜂常需發展對抗寄主包埋其卵之包囊反應，或藉對寄主齡期之偏好性以免遭受寄主之防禦反應 (Chien 1997)。臺潛蠅繭蜂為幼蟲至蛹期之體內寄生蜂，本文

表 4. 交尾頻率對臺潛蠅繭蜂雌蜂壽命、生育力及雌性比之影響

Table 4. Effect of mating frequency on longevity, fertility and female proportion of female *Opius caricivorae* [z]

		Longevity (d)		Fertility/female	
No. of mating	n	Female	Male	No. adults	Female proportion
0	11	19.3 ± 2.1 a [y]	–	213 ± 25 a	0.00 ± 0.00 b
1	12	21.9 ± 1.9 a	22.8 ± 1.3	277 ± 16 a	0.62 ± 0.03 a

[z] Female wasp was provided with 40–50 3rd instar larvae of *Liriomyza sativae* daily at 25℃.
[y] Mean ± standard error. Means within each column followed by the same letter(s) are not significantly different at 5% level by LSD test.

表 5. 不同蔬菜斑潛蠅密度時臺潛蠅繭蜂對寄主之利用率

Table 5. Effect of density of *Liriomyza* sativa on utilization of host by *Opius caricivorae* [z]

No. of host *L. sativae*	*n*	Host utilization (%) by wasp
1	10	80.0 ± 13.3 b [y]
2	9	100.0 ± 0.0 a
5	10	86.1 ± 4.8 b
10	9	82.2 ± 4.0 bc
20	8	90.0 ± 3.5 ab
30	8	62.9 ± 3.4 cd
40	8	59.4 ± 2.9 d

[z] The experiment was conducted in a glass cylinder (12 cm diameter × 21 cm height) at 25℃.

[y] Mean ± standard error. Means within each column followed by the same letter(s) are not significantly different at 5% level by LSD test. Data were arcsin-square-root transformed prior to analysis.

得知雌蜂產卵時寄主蔬菜斑潛蠅幼蟲會暫停不取食、蜂卵未被寄主細胞包囊及寄主齡期不影響其未成熟期之發育。Wan *et al*. (2005) 藉由臺潛蠅繭蜂寄生因子對寄主蔬菜斑潛蠅生理效應之觀察，推測蜂卵未被包囊之因，係雌蜂毒腺分泌之毒液抑制了寄主的包囊作用，至於類病毒顆粒是否參與此一過程尚不清楚。

Flanders (1942) 將寄生蜂卵形成之方式分為營養性胚胎膜 (hydropic) 與非營養性胚胎膜 (anhydropic) 二型。本文發現臺潛蠅繭蜂卵成熟時，其長度與寬度較初產時各增長 2.6 倍與 3.2 倍，進而得知其卵可藉胚胎膜自寄主蔬菜斑潛蠅體液獲得養分供胚胎發育，並證實其卵形成之方式為營養性胚胎膜，此論點與 Xu *et al*. (2007a) 觀察一致。另由該蜂之壽命、產卵期、日產卵數及取食寄主習性等特性，可歸納其產卵策略為應變式產卵 (synovigenic) 型式。

Yin *et al.* (2003) 與 Xu *et al*. (2007a) 曾進行臺潛蠅繭蜂成蜂生物學之初步研究，其部分成果與本文略同，但就成蜂交尾次數、幼蟲齡期及取食寄主之行為，則與本文有差異。如本文報導雄蜂一生交尾多達 8–17 次，4.5% 雌蜂交尾 2–4 次，而 Yin *et al*. (2003) 云僅雄蜂行多次交尾、雌蜂未發現 2 次交尾。本文依臺潛蠅繭蜂幼蟲頭寬可區分幼蟲有三齡，各齡頭寬之增長呈一定之比率 (第一與二齡頭寬比為 1.28，二與三齡頭寬比為 1.22)，符合 Dyar’s Law (Dyar 1890)。但 Yin *et al*. (2003) 與 Xu *et al*. (2007a) 則依臺潛蠅繭蜂幼蟲體長各區分幼蟲有四齡與五齡。Jervis & Kidd (1986) 謂寄生蜂雌蜂取食寄主之行為在膜翅目中十分普遍，已記錄者達 17 科、140 種。其行為模式有產卵取食併行但不殘害寄主 (concurrent non-destructive)、產卵取食併行但殘害寄主 (concurrent destructive)、產卵取食不併行但不殘害寄主 (non-concurrent non-destructive) 及產卵取食不併行但殘害寄主 (non-concurrent destructive) 四型。Chien & Ku (2001) 雖未觀察臺潛蠅繭蜂有取食寄主之行為，Yin *et al*. (2003) 則云被該蜂取食之寄主，有的可完成發育，有的死亡，本文證實該蜂雖具取食寄主行為，但不致死寄主，其取食寄主模式屬上述之第三型。

臺潛蠅繭蜂為行共育寄生性之寄生蜂，雖然寄主齡期不影響其未成熟期之發育 (表 2、3)，但寄生第一齡寄主時會顯著降低子代雌性比 (Chien & Chang unpublished data)。同時雌蜂交尾與否雖不影響子蜂數，但未交尾者子代均為雄性，無繁殖能力 (表 4)。因而建議該蜂繁殖時需注意寄主齡期與成蜂交尾。

臺潛蠅繭蜂為單員寄生蜂，但 Yin *et al*. (2003) 與 Xu *et al.* (2007a, b) 均發現該蜂有過寄生現象，後者更表明寄主蔬菜斑潛蠅密度可能是引起該蜂過寄生現象的原因之一。本文證實在寄主蔬菜斑潛蠅密度低時 (1、2、5 隻)，雌蜂過生率高達 66.5–87.5%，寄主密度為 10、20、30 及 40 隻時，雌蜂過生率即降至 0.9–9.2%，顯示該蜂確具辨識已被寄生寄主之能力。

害蟲生物防治時，害蟲各蟲期之寄生蜂均

表 6. 蔬菜斑潛蠅密度對臺潛蠅繭蜂在單隻寄主上不同產卵數機率與總產卵數之影響

Table 6. Effect of host density on normalized distribution of eggs per host and total eggs laid by *Opius caricivorae* [z]

No. host	*n*	Normalized distribution (%) of eggs per host									Total eggs/ female wasp
		No. wasp eggs/host									
		1	2	3	4	5	6	7	8	9	
1	8	12.5 ± 12.5 b [y]	0.0 ± 0.0 c	37.5 ± 18.3 a	0.0 ± 0.0 b	0.0 ± 0.0 a	12.5 ± 12.5 a	12.5 ± 12.5 a	12.5 ± 12.5 a	12.5 ± 12.5 a	4.4 ± 1.1 e
2	9	16.7 ± 8.3 b	16.7 ± 8.3 b	22.2 ± 12.1 ab	33.3 ± 8.3 a	0.0 ± 0.0 a	11.1 ± 7.3 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	6.3 ± 0.8 de
5	10	33.5 ± 11.1 b	41.2 ± 9.2 a	17.8 ± 5.5 ab	5.0 ± 5.0 b	2.5 ± 2.5 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	7.6 ± 0.8 cd
10	9	90.8 ± 4.7 a	7.6 ± 3.4 bc	1.6 ± 1.6 b	0.0 ± 0.0 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	9.0 ± 0.4 c
20	8	94.5 ± 2.1 a	5.5 ± 2.1 bc	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	19.1 ± 0.9 b
30	8	97.6 ± 1.2 a	2.4 ± 1.2 bc	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	19.9 ± 1.3 b
40	8	99.1 ± 0.6 a	1.0 ± 0.6 bc	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	24.0 ± 1.3 a

[z] The experiment was conducted in a glass cylinder (12 cm diameter × 21 cm height) at 25℃.

[y] Mean ± standard error. Means within each column followed by the same letter(s) are not significantly different at 5% level by LSD test. Data were arcsin-square-root transformed prior to analysis.

有其利用價值。在台灣，本地種蔬菜斑潛蠅幼蟲期之寄生蜂有六種，幼蟲至蛹期之寄生蜂一種 (Chien & Chang 2007)。其中底比斯釉小蜂 [*Chrysocharis pentheus* (Walker)] 與岡崎釉小蜂 [*Closterocerus okazakii* (Kamijo)] 已證實同為該蠅幼蟲期之有效寄生蜂 (Chien & Chang 2008a, b, 2009a, b)。本文就 25℃ 下蔬菜斑潛蠅每雌一生之生育力 (113 粒有活力卵數，Chien & Chang 2007)，與臺潛蠅繭蜂每雌一生所產子蜂數 (277 隻子蜂，表 4) 之比較，顯示臺潛蠅繭蜂對蔬菜斑潛蠅幼蟲至蛹期亦具強勢之抑制潛能。

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Morphology and Life History of *Opius caricivorae* (Hymenoptera: Braconidae)[1]

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Abstract

Chien, C. C. and S. C. Chang. 2012. Morphology and life history of *Opius caricivorae* (Hymenoptera: Braconidae). J. Taiwan Agric. Res. 61:144−157.

The wasp *Opius caricivorae* Fischer is an arrhenotokous, solitary endoparasitoid of the leafminer *Liriomyza sativae* Blanchard. The fertility of the female wasp is not affected by mating experience. Males mated many times (8–17 times) in a lifetime, but most females mated only once (4.5% mated 2–4 times). The premating period was 1.1 h. Female wasps fed on leafminers without killing them. The peak of emergence of wasps was between 0500 and 0800 h while the peak of oviposition was between 0500 and 0900 h. The development of wasps was not affected by the host instar. At 25℃, averaged duration for egg, larval, prepupal, pupal, and egg to pupal stage was 2.2, 5.0−5.1, 1.6−1.8, 5.9−6.1 and 14.9−15.0 days, respectively. Averaged survival from egg to pupal stage was 86.5−91.6%. When wasps were provided with 40−50 3rd instar larvae of *L. sativae* daily, the longevity of female and male wasps averaged 21.9 and 22.8 days, respectively. The fertility for a female wasp was 277 progenies. The ratio of female progeny was 0.62. Numbers of eggs, percentage of superparasitism and host utilization rate of wasps varied with host density. At low host density (1, 2 and 5 leafminers), the number of eggs per female wasp was low (4.4−7.6 eggs) and the percentage of superparasitism was high (66.5−87.5%), but at high host density (20, 30 and 40 leafminers), the number of eggs per female wasp was high (19.1−24.0 eggs) and the percentage of superparasitism was low (0.9−5.5%). Host utilization rates by wasps were high (80.0−100%) at low host density (1, 2, 5, 10 and 20 leafminers), however host utilization rates by wasp were low (62.9 and 59.4%) at high host density (30 and 40 leafminers).

Key words: *Opius caricivorae*, *Liriomyza sativae*, Morphology, Life history.

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Effect of Temperature on Prey Consumption Rate of the Green Lacewing *Mallada basalis* (Walker) (Neuroptera: Chrysopidae) to Two Species of Pest Mites, *Tetranychus kanzawai* Kishida and *Panonychus citri* (McGregor) (Acari: Tetranychidae)[1]

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Abstract

Cheng, L. L., J. R. Nechols, D. C. Margolies, J. F. Campbell, P. S. Yang, C. T. Lu, and C. C. Chen. 2012. Effect of temperature on prey consumption rate of the green lacewing *Mallada basalis* (Walker) (Neuroptera: Chrysopidae) to two species of pest mites, *Tetranychus kanzawai* Kishida and *Panonychus citri* (McGregor) (Acari: Tetranychidae). J. Taiwan Agric. Res. 61:158–164.

The green lacewing, *Mallada basalis*, has been shown to have high potential as a biological control agent against two mite species of economic importance on papaya: the Kanzawa mite, *Tetranychus kanzawai*, and the citrus mite, *Panonychus citri*. However, the impact that this predator can have on mite populations is dependent on prevailing environmental temperatures, which influence predator-prey dynamics by affecting developmental rate, survival, reproduction, and longevity. Another effect of temperature, which this study investigated, is predator consumption rate. We measured the consumption rates of 2nd instar *M. basalis* on *T. kanzawai* and *P. citri* at five temperatures (15, 20, 25, 30, and 35℃) in the laboratory. Results indicated that prey consumption by 2nd instar lacewings was temperature-dependent, increasing between 15 and 30℃ for both mite species; however, consumption declined at 35℃. Numbers of *T. kanzawai* nymphs consumed in a 30-min trial ranged from 16.3 ± 1.0 at 15℃ to 45.7 ± 2.5 at 30℃. On *P. citri*, predators consumed 26.4 ± 1.8 nymphs at 15℃ and 48.0 ± 3.3 at 30℃. There were slightly different trends in the consumption rate of the lacewing in response to the five temperatures between the two mites, and this might be the result of the different spatial distribution patterns of the two mites as well as different handling times of the lacewing for the two

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mites. High prey consumption rates across a wide temperature range, including temperatures as low as 15℃, suggest that *M. basalis* may be suitable for year-round application in Taiwan against both *T. kanzawai* and *P. citri*. However, further investigations on the effect of temperature on the predatory potential of *M. basalis* under field conditions are needed for effective application of this lacewing in papaya mite management programs.

Key words: Phytophagous mites, Predator-prey interaction, Feeding behavior, Predation rate, Biological control.

The Kanzawa spider mite, *Tetranychus kanzawai* Kishida, and the citrus red mite, *Panonychus citri* (McGregor), are two important acarine pests of a wide range of crops in many parts of the world (Bolland *et al*. 1998; Zhang 2003). They cause injury by penetrating plant tissue with their stylets to feed on cell chloroplasts. This results in leaf stippling, wilting, and defoliation, which ultimately reduces plant growth and productivity. Intensive application of miticides, combined with short life cycle and strong fertility of the mites, have led to the development of resistance to most registered miticides (Furuhashi 1994; Masui *et al*. 1995; Goka 1998; Aiki *et al*. 2005). Therefore, the search for alternative control measures has become essential.

Mallada basalis (Walker) is a common chrysopid in agricultural fields in Taiwan. Previous investigations have suggested its potential for controlling several species of arthropods. These include *Phyllocnistis citrella* Stainton, *Aphis* spp., *Nipaecoccus filamentosus* (Cockerell), *Diaphorina citri* Kuwayama, and *P. citri* on citrus; *T. urticae* Koch and *T. kanzawai* on strawberry; and *P. citri* on Indian jujube (Hao *et al*. 1996; Lo 1997; Hao 2002). A further benefit is that *M. basalis* can be mass-produced on a microcapsulated artificial diet in a cost-effective manner (Lee 1995, 2003). In addition, cold storage techniques have been established to store immature life stages for shipment and scheduled releases (Wu 1992). Finally, research has demonstrated tolerance in *M. basalis* to some insecticides, fungicides, and acaricides (Tzeng & Kao 1996; Lo 2002). All of these suggest that *M. basalis* is very compatible for use in integrated pest management.

Our earlier studies have provided further support that *M. basalis* has high potential as a biological control agent for both *T. kanzawai* and *P. citri* on papaya (Cheng *et al*. 2009, 2010, 2012). However, one factor that has not been investigated is how temperature influences predator-prey interactions between *M. basalis* and papaya mites. In general, temperature plays a crucial role in the bionomics of insects. Numerous studies have demonstrated effects of temperature on natural enemy development and population growth (Obrycki & Tauber 1982; Tauber *et al*. 1987; Lopez-Arroyo *et al*. 1999; Pervez 2004), as well as on foraging behavior, functional response, and predation capability (Runjie *et al*. 1996; Nielsen 1999; Mohaghegh *et al*. 2001; Flinn & Hagstrum 2002; Mahdian *et al*. 2006; Parajulee *et al*. 2006). This study investigated the effect of temperature on the consumption rate of *M. basalis* to two species of pest mites, *T. kanzawai* and *P. citri*, to provide information for understanding and effectively applying *M. basalis* in augmentative biological control programs for these two pest mites.

A laboratory colony of *M. basalis* was used for this study. This colony has been reared continuously, with no introduction of wild individual, since 1999 at 26 ± 2℃, 70 ± 10% RH and a photoperiod of 14:10 (L:D) on microcapsulated artificial diets developed for larvae (Lee 1994, 1995), and a Brewer's yeast and honey (1:1) diet for adults following the procedures described in Cheng *et al.* (2009). *T. kanzawai* and *P. citri* were collected from the papaya plantations in Nantou areas, and maintained on papaya seedlings at 26 ± 2℃, 70 ± 10% RH and a photoperiod of 14:10 (L:D) in separate rearing rooms. Papaya seedlings (*Carica papaya* L., 'Tainung No. 2') used for rearing the mites were purchased from a commercial nursery 3−4 weeks after germination. The seedlings were then transferred individually to 9-cm diameter pots, and maintained in a room at 26 ± 2℃, 70 ± 10% RH and a photoperiod of 14:10 (L:D) until they were approximately 25 cm tall and suitable for rearing mites. The seedlings were watered twice a week, but without any fertilization. For carrying out the experiments, a piece of papaya leaf

(~470 mm^2) with the undersurface facing up was floated on water (~4 mL) in a 5-cm diameter Petri dish. One hundred mite nymphs (either *T. kanzawai* or *P. citri*) were introduced onto the leaf and the unit was then placed in a growth chamber at 70 ± 10% RH and one of the following temperatures: 15, 20, 25, 30 and 35℃. One green lacewing second instar larva (at their 2nd day) which had been starved and kept in the growth chamber for 4 h prior to the test was then added onto the leaf in the Petri dish. The Petri dish was then covered with the lid. Thirty minutes later, the number of the mites consumed by the lacewing was recorded. Tests for each mite species and at each temperature were replicated at least 20 times. Because of a limited number of growth chambers, tests were blocked over time and the order was randomized for each block. Data were analyzed using the Kruskal-Wallis test because of non-normality of data distribution. Significantly different means were separated with Fisher's protected LSD procedures, with the significance level set at $P < 0.05$. All analyses were conducted using STATGRAPHICS Centurion XV software (StatPoint, Herndon, VA).

Results showed that prey consumption by 2nd instar *M. basalis* increased between 15 and 30℃, and then declined between 30 and 35℃ on both *T. kanzawai* and *P. citri* (Table 1). Except at the highest temperature (35℃), consumption was consistently higher on *P. citri* than on *T. kanzawai*. On *T. kanzawai,* the lacewing exhibited a statistically nonsignificant prey consumption rate between 15 and 20℃ (mean separation = 7.1; LSD = 7.8). Consumption then increased significantly with increasing temperature to 30℃ ($H = 47.05$; df = 99; $P < 0.0001$). Whereas on *P. citri*, the lacewing's prey consumption rate at 20℃ was significantly higher than at 15℃ (mean separation = 9.0; LSD = 8.15), but was similar between 20 and 25℃. Consumption then increased significantly between 25 and 30℃. In both species, the consumption rate decreased between 30 and 35℃, but the difference was only significant in *P. citri* (mean separation = 9.1; LSD = 8.15). The slightly different trend in consumption rate of *M. basalis* in response to temperature between the two mites may be due to the different spatial distribution patterns of the two mites, as well as the different handling times of the lacewing to the two mites (Cheng *et al*. 2009). *T. kanzawai* has a clumped distribution, but *P. citri* is more evenly distributed. This difference in spatial distribution between these two mite species is quite significant. At the lower temperature (e.g., 15℃), the lacewing might have a lower mobility. The effect of temperature on the lacewing's consumption rate might be less pronounced with species that have a clumped distribution because the lacewing does not have to move much to forage. This would assume that movement is limited to a greater degree by low temperature than prey handling. In an earlier study, we had demonstrated that the handling time of *M. basalis* with *P. citri* was shorter than with *T. kanzawai* (Cheng *et al*. 2009). Thus, at higher temperatures (e.g., between 30 and 35℃), the comparatively shorter handling times might make the effect of temperature on the consumption rate of *M. basalis* more pronounced with *P. citri*. The shorter handling time might also provide an explanation for the higher consumption rate of *P. citri* compared with *T. kanzawai* in this study. In addition, the effect of temperature on predator metabolism may also play a role in the consumption rates among various temperatures.

A number of studies have shown the relationship between temperature and various responses in predators, including green lacewings (Nee 1983; Ding & Chen 1986; Tauber *et al*. 1987; Nielsen 1999; Pervez 2004; Parajulee *et al*. 2006). For natural enemies in general, it has been suggested that increased feeding activity and search rates at high temperatures, as well as decreased handling times, enhance the functional response and, thus, have important impacts on the potential of natural enemies

Table 1. Prey consumption of *Mallada basalis* second instar larvae to *Tetranychus kanzawai* and *Panonychus citri* nymphs at various temperatures (total duration = 30 minutes) ($n = 20$)

Temperature (℃)	Mean (± SEM) number mites consumed	
	T. kanzawai	*P. citri*
15	16.3 ± 1.0 a [z]	26.4 ± 1.8 a
20	23.5 ± 1.9 a	35.4 ± 2.8 b
25	34.3 ± 2.4 b	37.5 ± 3.5 b
30	45.7 ± 2.5 c	48.0 ± 3.3 c
35	38.8 ± 4.7 bc	38.9 ± 2.9 b

[z] Means within the same column followed by the same letter are not significantly different at $P < 0.05$ (LSD = 7.80 and 8.15 for *T. kanzawai* and *P. citri*, respectively) (Fisher's protected LSD test; STATGRAPHICS Centurion XV, 2005).

to control target pests (Runjie *et al*. 1996; Mohaghegh *et al*. 2001; Flinn & Hagstrum 2002; Mahdian *et al*. 2006; Parajulee *et al*. 2006).

In a study with *M. basalis*, Chang (2000) indicated that the effective temperature range for larvae is about 15 to 43℃. The current study demonstrated that prey consumption by *M. basalis* increased within a narrower range of temperature, and that temperatures above 30℃ were not optimal for prey consumption. Although *M. basalis* exhibited lower predation rates at the lower temperatures of 15 and 20℃, at these temperatures both pest mites also have lower developmental rates. While *P. citri* has an optimal population growth rate at 25℃ (Zhang 2003), and *T. kanzawai* at 34℃ (Chang 2000), *M. basalis* showed increased predation rates at these temperatures. Our earlier studies have provided further support that *M. basalis* has strong potential as a biological control agent against both *T. kanzawai* and *P. citri* (Cheng *et al*. 2009, 2010, 2012). Based on the current study, it would appear that *M. basalis* is suitable for year-round application in Taiwan because mean monthly temperatures in Taiwan range between 16 and 30℃. However, our tests were conducted in small arenas and over a short period. Effects of temperature on the predator's search rate and the prey's ability to escape predation, and effects of prey spatial distribution on the predator's foraging efficiency, would be more prominent in field conditions. In addition, temperature fluctuations in the field may affect predator responses differently than the constant temperature conditions used for the laboratory investigation. Therefore, how temperature would affect the overall predatory potential of *M. basalis* in the field needs further investigation before this natural enemy can be effectively applied in pest management programs against mites.

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溫度對基徵草蛉 *Mallada basalis* (Neuroptera: Chrysopidae) 捕食神澤氏葉蟎 *Tetranychus kanzawai* 與柑桔葉蟎 *Panonychus citri* (Acari: Tetranychidae) 速率之影響 [1]

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摘 要

鄭玲蘭、James R. Nechols、David C. Margolies、James F. Campbell、楊平世、盧秋通、陳健忠。2012。溫度對基徵草蛉 *Mallada basalis* (Neuroptera: Chrysopidae) 捕食神澤氏葉蟎 *Tetranychus kanzawai* 與柑桔葉蟎 *Panonychus citri* (Acari: Tetranychidae) 速率之影響。台灣農業研究 61:158–164。

基徵草蛉 (*Mallada basalis*) 對神澤氏葉蟎 (*Tetranychus kanzawai*) 和柑桔葉蟎 (*Panonychus citri*) 兩種木瓜重要害蟎具有防治潛力，然而溫度會影響昆蟲的生長速率、存活、生殖和壽命，進而影響捕食性昆蟲與其獵物間的族群變動，因此溫度對草蛉壓制葉蟎族群之效能可產生重大影響。此外，溫度也會影響捕食性天敵對害蟲的捕食速率，本研究乃於實驗室內測定基徵草蛉於 5 種溫度下 (15、20、25、30 和 35℃) 對神澤氏葉蟎與柑桔葉蟎的捕食速率。試驗結果顯示基徵草蛉對兩種葉蟎之捕食速率於 15 至 30℃ 間隨溫度升高而增加，而溫度達 35℃ 捕食速率即降低。基徵草蛉之二齡幼蟲於 15℃ 下 30 分鐘內可捕食 16.3 ± 1.0 隻神澤氏葉蟎之若蟎或 26.4 ± 1.8 隻柑桔葉蟎之若蟎，而於 30℃ 下可捕食 45.7 ± 2.5 隻神澤氏葉蟎之若蟎或 48.0 ± 3.3 隻柑桔葉蟎之若蟎。基徵草蛉於 5 種試驗溫度下之捕食速率趨勢於兩種葉蟎間略有差異，可能與兩種葉蟎在木瓜葉片上有不同的空間分布，以及草蛉捕食兩種葉蟎所需的獵物處理時間 (handling time) 不同有關。基徵草蛉在廣泛的溫度範圍中對兩種葉蟎皆具極高的捕食效率，即使在 15℃ 的低溫下亦同，顯示基徵草蛉在台灣具有終年應用的潛力，但實際應用策略的擬訂仍待進一步田間試驗的評估。

關鍵詞：葉蟎、捕食性昆蟲-獵物交互作用、取食行為、捕食速率、生物防治。

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「台灣農業研究」稿約

一、 本刊爲行政院農業委員會農業試驗所發行之學術性季刊，刊登農業試驗研究相關之研究稿件：

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2、文獻須以英文撰寫，以姓名字母為序。作者相同時以年代先後為序。

3、文獻之書寫方法以期刊為例，依序為作者、年代、題目、期刊、卷期、頁數。

4、期刊名須依該期刊公告之縮寫撰寫；如無者，請參照 Biosciences Information Service (1992) 出版之 "Serial Sources for the BIOSIS Previews Database" 或依該學術領域之慣例撰寫。

5、文獻書寫範例如下：

(1) 原中文報告引用文獻出自學術性期刊 Hsu, S. H., T. Y. Chung, T. C. Kuo, and S. F. Wang. 1992. Studies on the micro-climate of greenhouse. I. Light environment of greenhouse. J. Agric. China 157:34–43. (in Chinese with English abstract)

(2) 原中文報告引用文獻出自著作性書籍 Sung, J. M. 1991. Nitrogen Metabolism of Rice. Chinese Agronomy Press. Taipei. 100 pp. (in Chinese)

(3) 原中文報告引用文獻出自編輯性書籍 Yang, C. M. and C. Y. Lin. 2003. Studies on rice precision farming system. p.1–12. *in*: Rice Precision Farming System. (Yang, C. M. and C. Y. Lin, eds.) Agric. Res. Inst. Pub. No. 105. Taichung. 252 pp. (in Chinese with English abstract)

(4) 原中文報告引用文獻出自研討會專刊 Chang C. A. 2001. Application and extension of virus-free seedling for the control of virus diseases of passionfruit in Taiwan. p.31–38. *in* the Proceeding of Symposium on Application of Healthy Plant for Control of Plant Diseases. Taiwan Phytopathol. Soc. Pub. Taichung. (in Chinese with English abstract)

四、 單位、縮寫格式、括號及統計分析

1、凡文中出現之數值，除非另有特別之意義，一律用阿拉伯數字書寫。單位如以英文表示，則用國際公認標準符號 (SI units) 代替全文，各常用之縮寫單位參照說明如下：

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(4) 面積：公頃 (ha)、平方公尺 (m^2)、平方公分 (cm^2)。

(5) 溫度：攝氏 (℃)、絕對溫度 (K，但須加以說明)。

(6) 時間：日 (天) (d)、時 (h)、分 (min)、秒 (s)。

(7) 濃度：莫耳 (M)、千分之一莫耳 (mM)、當量 (N)、千分之一當量 (mN)。

(8) 重力加速度：*g*。

2、其他之縮寫：平均 (av)、大約 (ca)、直徑 (diam.)。

3、括號：括號字型全以半形 ( )，括號前後各空一格。

4、統計分析：達顯著性水準以 *、**或處理間顯著差異區分，以 a、b、c、…表示者均應加註顯著機率值 P (例如 P < 0.01)；各統計量請採慣用符號，例如相關係數 (r) 、決定係數 (R2)、變異係數 (cv)、標準差 (sd)、平均值標準差 (se) 等。

5、其他問題：

(1) 單位與其前面數值之間應空一格，如 220 g。

(2) 數學運算符號與其前後數值之間應各空一格，如 P = 0.014、23.6 ± 3.0、15 × 2。

(3) 在±、×或 to的前後兩數值若是含有單位，請用 23.6% ± 3.0%、15 cm × 2 cm、20°C to 30°C (即勿省略前面數值之單位)。

(4) 數值起迄符號用 – (全型破折號)，勿用 ~或-，且與前後兩數值之間無須留空格，如 3–6、2005–2007年、7月 20–25日。

(5) 年代一律用西元。

五、 其他注意事項

1. 生物學名以斜體字表示，屬名第一字母大寫。學名於摘要、本文、每一圖表中第一次出現時，須寫全名，如 *Citrus sinensis*；第二次出現時，屬名保留第一字母後加句點 “.” ，種名全寫，如 *C. sinensis*。生物學名於本文中首次出現時應加註命名者。

2. 中文稿件中，外文名詞應儘量翻譯成中文，若是不常見名詞應於文中第一次出現時以括號標出原文。摘要及本文中，英文縮寫於第一次出現時，須附全名。

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